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CYTOCHEMICAL STUDIES OF PLANETARY MICROORGANISMS EXPLORATIONS IN EXOBIOLOGY

Final Report Covering Period April 1, 1960 to May 30, 1980
For
National Aeronautics and Space Administration

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Instrumentation Research Laboratory, Department of Genetics Stanford University School of Medicine Stanford, California 9:305

FINAL REPORT

CYTOCHEMICAL STUDIES OF PLANETARY MICROORGANISMS

EXPLORATIONS IN EXOBIOLOGY

A Program of Research at Stanford University*

Covering Period April 1, 1960 to May 30, 1980

Prepared by: Elliott C. Levinthal, Ph.D.

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Joshua Lederberg, Principal Investigator
April 1, 1960 - October 1, 1978

Elliott C. Levinthal, Principal Investigator October 1, 1978 - May 30, 1980

^{*} Retitled "Molecular Evolution in Primitive and Simple Biochemical Systems" September 9, 1977

Preface

This report is an historical overview of the activities at Stanford University supported by this grant. The grant's original title was: "Explorations in Exobiology - Cytochemical Studies of Planetary incoorganisms, A Program of Research at Stanford University." The report also briefly discusses relevant work supported by other resources which either contributed to the goals of this grant or which were an outgrowth of this work.

For details of the research the reader is referred to the annual reports and the publications that originated from this grant.

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LIST OF IRL REPORTS REFERENCED IN FINAL REPORT

Number	Title
1003	Status Report Covering Period March 1, 1962 - April 1, 1963
1010	Fluorescein and Naphthol Substrates for Phosphatase Assays by J. Westley
1013	Status Report Covering Period April 1, 1963 - March 31, 1964
1014	Video Tracking Technique In Biological Research by N. Veizades
1027	Status Report Covering Period April 1, 1964 - September 1, 1965
1035	An Investigation of the Mass Spectra of Twenty-Two Free Amino Acids by N. Martin
1036	DENDRAL-64: A System for Computer Construction, Enumeration and Notation of Organic Molecules as Tree Structures and Cyclic Graphs submitted by Joshua Lederberg
1042	The Stereospecificity of @-Chymotrypsin-Catalysed Hydrolysis and Alcoholysis of Specific Ester Substrates by B. Halpern, J. Ricks and J.W. Westley
1050	Status Report Covering Period September 1, 1965 - April 1, 1966
1054	Status Report Covering Period April 1, 1966 - September 30, 1966
1056	Status Report Covering Period October 1, 1966 - April 1, 1967
1061	Status Report Covering Period April 1, 1967 - October 1, 1967
1062	A Computer Operated Mass Spectrometer System by W.E. Reynolds, T.B. Coburn, J.C. Bridges and R.B. Tucker
1076	Status Report Covering Period October 1, 1967 - March 31, 1968
1082	Status Report Covering Period April 1, 1968 - September 30, 1968
1087	Status Report Covering Period October 1, 1968 - December 31, 1968
1091	Status Report Covering Period January 1, 1969 - March 31, 1969
1092	Summary Report Covering Period July 1, 1968 - July 1, 1969
1105	Status Report Covering Period July 1, 1969 - January 1,1970
1110	Summary Report Covering Period July 1, 1969 - July 1, 1970
1123	Status Report Covering Period July 1, 1970 - January 1, 1971
1128	Status Report Covering Period January 1, 1971 - December 31, 1971
1158	Status Report Covering Period January 1, 1972 - December 31, 1972

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LIST OF ATTACHMENTS

Attachment

- 1. March 4, 1959 letter from Lederberg to Jastrow.
- 2. March 25, 1959 letter from Lederberg to Schilling (with attachments).
- 3. December 10, 1959 letter from Lederberg to Jastrow.
- 4. October 10, 1959 memo from Lederberg to SSB: "Ten Year Program in Space Science".
- 5. "Exobiology, Experimental Approaches to Life Beyond the Earth" paper given by Lederberg at First Intl.
 Space Sciences Symposium, January 1960.
- 6. January 28. 1960 letter from Jastrow to Lederberg.
- 7. February 2, 1960 letter from Lederberg to Newell (with tentative budget proposal attached).
- 8. February 10, 1960 letter from Newell to Lederberg.
- 9. March 11, 1960 letter from Randt to Lederberg.
- 10. March 30, 1960 letter from Lederberg to Willner (with budget proposal attached).
- 11. Memo "Status of Exobiology Program" dated September 4, 1960.
- 12. Report to NASA for period April 1, 1960-March 31, 1961.
- 13. First Progress Report, submitted September 4, 1960.
- 14. Medical Center Memo, Vol. 3, No. 2, January 25, 1961.
- 15. "Explorations in Exobiology Cytochemical Studies of Planetary Microorganisms: A Program of Research At Stanford University"
- 16. November 4, 1960 letter from Lederberg to Newell (with attached remarks).
- 17. Proposal to NASA for period April 1, 1961-March 31, 1964.
- 18. Report to NASA for period April 1, 1961-February 28, 1962.
- 19. May 1, 1961 letter from Lederberg to Johnson.

LIST OF ATTACHMENTS (cont'd.)

Attachment

- 20. May 12, 1961 letter from Lederberg to Newell (with Multivator proposal attached).
- 21. February 16, 1962 letter from Bowker to Smull (with Biomedical Instrumentation Laboratory proposal attached).
- 22. Memorandum of Understanding between NASA and Stanford.
- 23. Medical Center Memo, Vol. 4, No. 15, October 10, 1962.
- 24. August 3. 1966 memo from Levinthal to Lindee.
- 25. October 18, 1966 letter from Smull to Pettit.
- 26. February 6, 1967 letter from Sterling to Webb.
- 27. "An Instrumentation Crisis in Biology" by Lederberg, Appendix C of IRL Report 1003.
- 28. Proposal to NASA for Supplement for period April 1, 1963-March 31, 1964.
- 29. STANFORD TODAY, Winter 1963.
- 30. Medical Center Memo, Vol. 5, No. 14, September 15, 1963.
- 31. "Signs of Life: A Survey of the Detection Problem in Exobiology" by Lederberg.
- 32. "Signs of Life: Criterion-System of Exobiology" by Lederberg, Nature, Vol. 207, No. 4992, pp. 9-13, July 3, 1965.
- 33. "Some Comments on Instrumentation Design Strategy for Exobiological Explorations" by Levinthal.
- 34. "A Pasteur Probe: A Proposed Experiment" prepared by Lederberg.
 Tecnical Report IRL 1016.
- 35. Research and Technology Resume for NASA.
- 36. Non-technical memo from Levinthal to Young.
- 37. Stanford M.D., Fall 1974, "Are There Microbes on Mars?"
- 38. Research and Technology Resume for NASA, June 23, 1977.

Introduction

Since Professor Joshua Lederberg's ideas were the seminal force that underlay the origin and growth of all these activities, it is appropriate to start this historical review with two memos that illustrate his early interest in the space program. This first memo expresses his concerns with contamination policy.

LUNAR BIOLOGY?

If the launching of a moon-bound satellite is within the imminent reach of present day technology, biologists should join with other scientists in giving some forethought to the implications of this accomplishment for their own science. That little or nothing has been said about this subject so far may be put down on the one hand to the probability that lunar conditions are incompatible with any form of continued life, on the other to the too visionary context of such speculations.

However, if a lunar satellite is a near reality in fact, it is unrealistic not to anticipate it.

There is at least one important issue that requires advance planning if a unique opportunity is not to be confounded or lost. This is whether or not the meteoritic dust which supposedly covers the surface of the moon contains viaible spores. Although the most likely origin of such spores would be terrestrial escapes, e.g. from volcanic catastrophes, the possibility of a small fraction of them remaining viable would be the most direct empirical test of Arrhenius' biospore theory which hitherto has had to remain befogged by pure speculation.

Without forethought, satellites may be sent to crash on the moon—without any care to eliminate or minimize the artificial contamination by bacterial spores, etc., that they may carry with them. It will probably be many years longer before we can retrieve samples from the moon's surface, and by then it may no longer be possible to judge whether spores can achieve as well as survive interplanetary transit by natural agencies.

This is only one issue; others may arise. My principal conclusion is that the responsible agencies of the U.S. and other governments should have the advice of an expert committee to deal with questions of extraterrestrial biology, as they already have on innumerable aspects of the physical sciences.

Joshua Lederberg University of Wisconsin December, 1957 3

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* Dr. Lederberg joined the Stanford faculty in February 1959.

The second retrospective memo, dated January 2, 1976, recalls his early interests.

MEMO ON INITIAL INTEREST IN PLANETARY QUARANTINE

I thought I had committed this to type long since but failing to find that purported record, I thought I had better dictate a fresh note.

Sputnik was launched on October 4. 1957. At that time I was visiting Melbourne on a Fulbright lectureship. As it happens, Sputnik was for some time observable only from the southern hemishere and we were lucky to get an early look at it within a day or so of its launch. The excitement that this event generated throughout the world is well documented in the press and corresponds to my own vivid recollection. I had, I should stress, long since been interested in speculations about planetary life in the light of their relationship to the problem of the origin of terrestrial life. So, I had been following the course of rocket technology with some moderate but not intense interest up to that point. It seemed obvious to me that this launch would be followed very, very quickly by rapid further technological progress and I began to think immediately of the implications for planetary traffic.

In early November we left Australia on an itinerary that would take us to visit Haldane in Calcutta for a round-the-world return to Madison, Wisconsin.

Fortunately, I have been able to find a letter to Haldane (dated February 1959) which helps to document my personal recollection of that experience. I also have a fragment of another letter to James F. Crow which corroborates that impression. These documents are attached.

The night of our arrival was the occasion of a lunar eclipse which was regarded as an important religious festival in Calcutta. It was also the occasion for a good deal of dinner table conversation (besides the Haldanes, Rana Mahalanobis, there were one or two other guests including a herpetologist, I believe, called Patterson). Many members of the group were quite strongly pro-Soviet in their inclinations and they were almost gleeful at the prospect that the Soviet Union would follow up its October 4th triumph with another launch perhaps even directed at the moon during the lunar eclipse. So, we even stayed up to see if there would be such a demonstration although we were well aware of the physical difficulties of arranging for something that could be visible from earth. That occasion led me to think very sharply about the extent to which political motives would outweigh scientific ones in the further development of the space program - which is of course precisely what has happened to a very large measure.

As soon as I returned to Madison, I began to think what ought to be done about the situation and I drafted a number of letters and memoranda, eventually directing them to the National Academy of Sciences, urging a more deliberate look at the problem. These documents are also attached.

The consequences of these initiatives, as are well documented now in the official history, were the establishment by NAS of an official position on the question of quarantine, some encouragement to the formation of Cetex, and some support for the establishment of one and then later two committees in the U.S. which came to be called Westex and Eastex because of their geographical locales. (This was very much at my own initiative since I was as uninterested in frequent transcontinental travel then as I am today.)

These events preceded the official reconstruction of NASA from the NACA, which tended to give the NAS a remarkable degree of influence in establishing our preliminary policy.

Once these committees were established I did not feel there was a great deal more that I needed, or indeed would be able to do. about the situation; but I did accept membership on a number of the related panels. In

due course I felt that I had to respond to the challenges of planetary bilogy in a more constructive way than merely to attempt to dampen exploration in the name of the contamination problem. So I have been a member of advisory groups to NASA since that time, including the Space Science Board; I have also undertaken technological development programs in my own laboratory that were intended to lay the groundwork for planet—based biological investigation and to assist in offering wise critical counsel about national policies. In that vein I have been a member of the Mariner and now of the Viking experimenter teams.

In addition, the technological orientation of these efforts — which was very different from my scientific work up to that time — led in a very direct fashion to my interests in the uses of computers in scientific methodology and eventually to my current interests and activities in artificial intelligence.

Joshua Lederberg Chairman and Professor Department of Genetics Stanford University JANUARY 2, 1976 3

I. Early History

1959

The first expression of interest in the possibility of receiving support from NASA for work in the Genetics Department at Stanford University is contained in a letter to Dr. Robert Jastrow, Chief, Theoretical Division, NASA, dated March 4, 1959 (Attachment 1). This letter expresses interest also in getting some help from a young astronomer at Yerkes-Madison named Carl Sagan.

Correspondence with Dr. G.F. Schilling, Chief, Astronomy and Astrophysics Program, NASA, dated March 25, 1959, included as an attachment notes entitled "Preliminary Studies in Planethry Biology" (draft copy—Attachment 2). These contained a further elaboration of the notion that Stanford's Genetics Department might become involved in the space program. This was responded to by a letter from Nancy G. Roman, Head, Observational Astronomy Program, Office of Space Science, NASA, asking for a more detailed specific program.

Correspondence with Jastrow, dated December 10, 1959 (Attachment 3: This refers to a memorandum of November 25 which is not found in our files) mentions the difficulties that NASA has organizing its exobiological program. Lederberg had requested \$25,000 from the Rockefeller Foundation and received \$10,000 for preliminary support. Lederberg expresses the hope that NASA support will start in fiscal 1961 at a level of \$40,000 per year through fiscal 1963.

These activities parallel a NAS developing involvement with the Space Science Board on the part of Professor Lederberg.

Lederberg was a founder of WESTEX, a group of West Coast biologists interested in the biological exploration of the planets. This group complemented a similar group centered at Massachusetts Institute of Technology (EASTEX). These groups convened at the request of Dr. Bruce Rossi of the Space Science Board (SSB). The MIT group met December 4 and 19-20, 1958. WESTEX met February 21, March 21, May 2-3 and in September of 1959. The primary initial concern was policy questions dealing with the problems of contamination and planetary quarantine and to provide advice to the SSB in preparation for meetings of CETEX and COSPAR at the Hague, March 9, 1959. The groups also considered the constructive aspects of research in exobiology. The quarantine issues and the history of their consideration are reviewed in a book edited by Lawrence B. Hall "Planetary Quarantine", Gordon and Bracher, 1971. (This material also appeared in Vol. 1, No. 1 and 2, 1971, Environmental Biology and Medicine.) The summary report of WESTEX, February 21, 1959 to September 1959, to the SSB of NAS contains an interesting discussion of the experimental aspects of exobiology. Memorandum SSB 93. dated May 27, 1959, gives the ideas of EASPTX generated at their December 1958 meetings regarding the problems of detecting extraterrestrial life.

A memorandum to the SSB, submitted October 10, 1959 by Professor Lederberg, "Ten Year Program in Space Science", is included as Attachment 4.

The general principles of exobiology as an experimental science were laid down in a paper given by Lederberg at the First International Space Science Symposium sponsored by COSPAR (Committee on Space Research) held at Nice in January 1960 (Attachment 5).

Following conversations at Nice, Jastrow expressed strong support for developmental work on planetary biology experiments in his letter to Lederberg dated January 28, 1960 (Attachment 6). This was followed by a letter from Lederberg to Dr. Homer Newell, Deputy Director for Space Scienve, NASA, dated February 2, 1960 (Attachment 7) which included a tentative budget for fiscal 1961 of \$40,875 and an outline of a proposal entitled "Cytochemical Studies of Planetary Microorganisms."

This elicited an immediate response from Newell (Attachment 8) on February 10, 1960, stating that he was forwarding Lederberg's letter to the appropriate officer for action. On March 11, Lederberg received a letter from Dr. Clark T. Randt, Director, Office of Life Sciences (Attachment 9) stating that the grant application had been favorably considered and that formal approval would be forthcoming. This was followed by a letter on March 30, 1960, to Mr. William Willner, Procurement Officer, NASA (Attachment 10), with a formal budget of \$37,605 for the period April 1, 1960 to March 31, 1961 based on the work outlined in the February 2, 1960 letter to Newell (Attachment 7).

On April 15, Ernest W. Brackett, Contracting Officer, notified the University that Research Grant NSG-81-60 under the direction of Dr. Joshua Lederberg was awarded in the amount of \$37,605 for the period April 1, 1960 to March 31, 1961. This was accepted for the University by Prof. Albert H. Bowker, Dean, Graduate Division, on August 5, 1960.

At a meeting of the SSB on June 25, it was decided to consolidate advisory activities of the National Academy of Science in space research. This was done by a consolidation of the Bioastronautics Committee within the SSB. Committee 14: Exobiology, a successor to the former committee 11, was organized with the same membership as WESTEX, including Prof. Lederberg. At the same time NASA organized the Pioscience Advisory Committee to review operational proposals.

An interesting memo prepared by Lederberg is included as an attachment. The memo "Status of Exobiology Program", September 4, 1960, places a strong emphasis on microbiology in planetary investigations (Attachment 11).

II.

A. April 1, 1960 - March 31, 1961 (A report covering this period is included as Attachment 12)

During this preliminary period the initial staff was recruited and work was started on the development of a fast scanning microspectrophotometer using a digitized vidicon camera control. The concept was to observe the differential absorption of particles in the microscopic field, microorgannisms having characteristic absorption maxima in the region of 260-280 milimicron.

The first progress report on work at Stanford in exobiology was submitted September 4, 1960. This is included as Attachment 13 and contains an outline of a prototype for Martian soft landing.

Attachment 14 (Medical Center Memo, Vol. 3, No. 2, January 25, 1961) is a news release giving some views of this early work.

The concept of the Multivator was also under development with work started on sensitive assays for the phosphatase enzyme. The Multivator was submitted as a proposal for a Mariner B Experiment Capsule.

Efforts were also initiated on methods of microbial concentration.

These ideas were discussed in a document (Attachment 15)
"Explorations in Exobiology, Cytochemical Studies of Planetary Microorganisms:
A Program of Research at Stanford University". A letter to Homer Newell,
November 4, 1960 (Attachment 16), stresses the urgency in developing
scientific hardware to carry out simple biological experiments.

Dr. Elliott Levinthal joined the staff as Technical Manager in February of 1961.

On November 30, 1960, an application for renewal and increase of scope of the grant was submitted to Dr. Freeman H. Quimby, Assistant Director, Life Sciences, NASA. The amount requested was \$386,200 for a three year period April 1, 1961 to March 31, 1964. This was subsequently revised to \$380,640. The grant was awarded March 1961.

The proposal (Attachment 17) focused on projected landings on Mars in the period 1964-67. The research was to be directed toward basic problems in the detection of microorganisms and their characterization with regard to the role of nucleic acids and proteins. It was anticipated that there would be benefits to terrestrial applications in science and medicine. The specific efforts on "Multivator" were to be coordinated with a NASA laboratory such as JPL.

The possibility of collaboration with a NASA Life Science Laboratory that might be established in the Bay Area was also discussed.

B. April 1, 1961 - February 28, 1962

Attachment 18 is a report covering this work.

This period was characterized by intensive work on the Multivator. This involved collaboration with George Hobby and Jerry Stewart at JPL. The conceptual notion was that the biological scientists would have a simpler interface to carry out space research: individual modules in a Multivator rather than the national space program. It is interesting to note that among the experiments considered possible to include in the Multivator were C^{14} labelled nutrients and the observation of labelled CO_2 by selective permeation as well as measurement of CO_2 fixation. A great deal of work was done on the phosphatase catalyzed hydrolysis of α -naphthol phosphate and the use of fluorescein as a substrate.

The electronics for a high-speed u-v microspectrophotometer were designed and constructed. Two engineers, Lee Hundley and Harrison Horn and a microbiologist. Dr. Larry Hochstein, joined the project.

In April of 1961, President Kennedy asked Vice President Johnson to head a study to seek an area in which the U.S. can have a pioneering role. A letter (Attachment 19) to Johnson from Lederberg urges consideration of the Mariner B, a combined fly-by and Mars entry capsule.

On May 12, 1961, Lederberg submitted to H. Newell, NASA, in collaboration with George Hobby and Jerry Stewart of JPL, a proposal for "Multivator" to be included in the Mariner B capsule (Attachment 20).

On February 16, 1962, the University submitted an aplication to T.K. Smull of NASA for a facility grant of \$ 0.000 based on a proposal prepared by Lederberg and Levinthal (Attachment 21). These negotiations were concluded October 2, 1962, and resulted in a memorandum of understanding between Dr. J.E. Wallace Sterling, then President, Stanford University and James E. Webb, Administrator, NASA (Attachment 22), Vol. 4, No. 15, October 10, 1962 of the Medical Center Memo (Attachment 23) features the public announcement of this grant. Occupation of these laboratories occurred in the beginning of 1966. The formal dedication ceremonies took place on May 26, 1966. A memo from Levinthal to Robert G. Lindee, August 3, 1966 (Attachment 24) outlined the initial steps that were taken to implement the memorandum of understanding. James Webb visited Stanford on October 29, 1966. Among the purposes of this visit (see letter October 18, 1966 of Smull to Dean Joseph M. Pettit. Attachment 25) was a review of the implementation of the memorandum. President Sterling's letter of February 6, 1967 to James Webb (Attachment 26) outlines the accomplishments in carrying out the purposes of the memorandum.

C. MARCH 1, 1962 - APRIL 1, 1963

During this period, the Instrumentation Research Laboratory (IRL) was organized as a part of the Department of Genetics. The work during this period is covered by IRL Status Report 1003.

Lee Hundley completed the breadboard design of the Mark I multivator. Collaboration was initiated with Prof. J. Arnold in the Mechanical Design Department, Stanford University. This work was supported in part by this grant and in part by a contract with JPL (Contract 950-535).

Work was continued on the fluorescent photophatase assays.

J. Lundstrom joined IRL and started investigations of membrane separation techniques that might be useful if a Gulliver-type experiment as proposed by G.V. Levin were incorporated in the Multivator.

Further work continued on video scanning techniques. This closely related to work on A.F. Contract AF18(600)1911 which had commenced June 1961 and continued until November 1964. This contract studied the application of video tracking techniques to the recognition of motile microorganisms in microscopic fields containing a large background of stationary particles. This work was largely carried out by N. Veizades who joined IRL in November 1962. This work is reported in detail in IRL Technical Report 1014, November 1, 1964, by Nicholas Veizades.

Increased interest was expressed in exploiting the possibilities of computer technology as applied to the problem of exobiology. Collaboration was initiated by Prof. J. McCarthy of the Computer Science Department with arrangements to share access to a PDP-1 computer. IRL was selected to receive one of the computers in the LINC Evaluator Program. This general interest and the desire to compare a time-shared system (PDP-1) with a stand-alone computer (LINC) is expressed in a report entitled "An Instrumentation Crisis in Biology" (see Attachment 27, Appendix C of the IRL Status Report 1003). Digital microprocessors are becoming routine just now in bioinstrumentation design.

During this period the biochemistry group was joined by Dr. Elie Shneour (October 1962) and Dr. John Westley(September 1963). Dr. Hochstein left IRL to join Ames Research Center, NASA. Dr. Morton Mandel, a physicist, joined IRL in April 1963.

Discussions concerning renewal of the grant for the period April 1964 to June 30, 1966 were carried out with Drs. Orr Reynolds and Freeman Quimby of NASA Hdq. A supplement of \$132,000 was requested and granted for the period April 1963 to March 31, 1964 (Attachment 28). This was to allow expansion of the program to the level envisaged under the NASA facilities grant (NSG [F]-2) and the purchase of image-reading components to be used with the PDP-1 computer.

D. April 1, 1963 - September 1, 1964

The period April 1, 1963 to March 31, 1964 is covered by IRL Status Report 1013.

This report period is marked by the publication of the monograph "Computation of Molecular Formulas for Mass Spectrometry" by J. Lederberg. This application of computers to high resolution mass spectrometry formed one of the major underpinnings of DENDRAL which has become one of the most successful application of Artificial Intelligence techniques, certainly in the

chemical domain, and which has spawned a host of important research activities.

John Westley reported on stable and sensitive flourescein and napthol substrates for phosphatase assays (IRL Report 1010) and Jerry Lundstrom continued his work on membrane separation (IRL Report 1012).

Dr. Barthold Halpern joined the Biochemistry Group of IRL in August 1964 and William Bonner the Engineering Group in November of 1963.

A paper on the Multivator was presented at COSPAR in Warsaw. June 1963, by Levinthal in addition to numerous other presentations and lectures on the Biological Exploration of Mars.

The Winter 1963 issue of "Stanford Today" (Attachment 29) was devoted to exobiology. It contained an article by Lederberg "Life Beyond the Earth" and one by Levinthal "Payload to Mars", describing the Multivator.

A public release on the Multivator is contained in Medical Center Memo Vol. 5. No. 14. September 15, 1963 (Attachment 30).

Dr. Morton Mandel, using the NMR facilities at Varian Associates, studied the magnetic resonance of compounds of biological interest. These included 200 common l-amino acids which were related to studies of spectra of ribonuclease, lysozyme and cytochrome C. Some of the first studies of phosphorous chemical shifts in polynucleotides as a means of end-group analysis of short chains were also carried out.

Dr. Levinthal served as a member of the Biosciences Subcommittee of NASA. Prof. Lederberg, Drs. Shneour and Levinthal were members of a Steering Committee that directed an Exobiology Summer Study. Prof. Lederberg with Prof. Colin Pittendrigh served as co-chairmen of this study carried out under the auspices of the Space Science Board of NAS. This resulted in two books: "Biology and the Exploration of Mars", Publication 1296, NAS, NRC, 1966, and "Extraterrestrial Life: An Anthology and Bibliography", Publication 1296A, NAS, NRC, 1966.

Further work with the Mechanical Enginering department continued. On June 16, 1964 a supplemental award of \$62.984 was made to support "Investigation into the Mechanical Design and Miniaturization of a Multi-Chamber Life Detector." This covered work for the period February 1, 1964 to January 31, 1965 under the direction of Professor Peter Z. Bulkeley.

On February 28, 1964, a proposed continuation of the grant was submitted with a budget of \$485,120 for a one year period beginning April 1, 1964. This was approved May 28, 1964.

This proposal initiated intimate collaboration with Dr. Djerassi's mass spectrometry laboratory in the Chemistry Department. This collaboration contributed to the generation of many important papers listed in the annual reports of this grant. The budget included funds for the purchase of an Atlas CH-4 instrument.

The proposal included two memoranda as attachments. One entitled "Signs of Life: A Survey of the Detection Problems in Exobiology" by Prof. Lederberg (Attachment 31) was the precursor of the paper "Signs of Life: Criterion-System of Exobiology", J. Lederberg, Nature, Vol. 207, No. 4992, pp. 9-13, July 3, 1965 (Attachment 32). The second was entitled "Some Comments on the Design Strategy of Instruments for Exobiological Explorations" by Elliott Levinthal (Attachment 33).

A proposal to establish PLANETLAB as a spacecraft resource facility was made by Lederberg on 12/7/63 to Dr. Orr Reynolds and the Bioscience Subcommittee. PLANETLAB was to be assigned for development to a NASA laboratory or a subcontractor.

E. September 1, 1964 - September 1, 1965

IRL Report 1027 covers the period April 1, 1964 to September 1, 1965.

A report was submitted entitled "Multivator Design Studies" in September of 1965. The design work on a "one-step" multivator system was carried to the point which would permit rapid development of a final design specification should a mission requirement arise for such a device. A laboratory test unit was designed to study problems of a complex sequence associated with multi-step chemical processing. This accomodates a heat sterilizable binary substrate, stored in two separate components developed in IRL for the phosphatase assay. Optical design studies for miniaturized fluorometric assays were also carried out.

Based on extensive work carried out by the Chemistry group under Dr. Halpern (see, for example, "Optical Resolution of D.L Amino Acids by Gas Chromatography and Mass Spectrometry, B. Halpern, J.W. Westley, I. von Wredenbergen and J. Lederberg, Biochemical and Biophysical Res. Com. Vol. 20, No. 6, 1965), a proposed experiment, "A Pasteur Probe" (IRL 1016 [Attachment 34]) was submitted to NASA on March 10, 1965.

The Atlas CH-4 was installed in Dr. Djerassi's laboratory and played a role in producing seven papers related to natural products.

A program was started on mass spectrometry image scanning modeled after a prototype by G. Slodzian and R. Castaing. Dr. Sidney Liebes Jr. headed this effort and was joined by Dr. G. Slodzian from the University of Paris for the initial development effort.

The fundamental work on computerized mass spectrometry data interpretation led to the development of a language for representing organic molecules as free structures, hence dendritic algorithm. DENDRAL-64 (see J. Lederberg, DENDRAL-64. A System for Computer Construction, Enumeration and Notation of Organic Molecules as Free Structures and Cyclic Graphs. Part 1" NASA CR-57029 STAR No. N65-13158, IRL 1036, 1964 and J. Lederberg "Topological Mapping of Organic Molecules" Proc. Nat. Acad. Sci. U.S. 53: 134-139 [1968]) were the first in a continuing series of papers and reports representing work that has continued to flourish.

In connection with the work on mass spectrometry a Bendix Time-of-Flight (TOF) instrument was acquired and, as a method of gaining insight into the problems of computer control, was used to gather a comprehensive set of spectra of amino acids from solid samples. This was jointly supported by this grant and National Institute of Neurological Diseases and Blindness Grant NR-04270 and and Air Force Grant AF-AFOSA-886-65. The results were submitted in IRL Report 1035.

A proposed continuation of the grant was submitted January 20, 1965, requesting \$349,899 for the period of one year beginning April 1, 1965. This included \$52,469 as funds for the Mechanical Design Department subtask under Prof. Bulkeley.

This proposal deemphasized the further development of Multivator. The emphasis was on three areas: mass spectrometry, tests for the separation of asymetric carbon centers, and fluorometry.

This proposal was accepted April 12, 1965.

F. September 1, 1965 - April 1, 1966

This period is covered in IRL Status Report 1050.

A new neon light source was developed for the Multivator for use in the detection of 6.6^{1} dihydroxy-napthofluoran (N.F.). This gave a 100-fold improvement to yield a lower limit of detection close to 10^{-10} molar concentration of N.F.

Work continued with Dr. Lubert Stryer of the Department of Biochemistry on the development of a fluorogenic substrate that could function for a broad array of enzymes.

Also in collaboration with Dr. Stryer a nanosecond flash fluorometer was developed in IRL. This work is described in a progress report under Grant NGR-05-020-137 by Profs. A. Kornberg and L. Stryer. A major continuing research activity on the part of Prof. Stryer has developed from this initial effort. The following three references describe some of the results of this effort.

Yguerabide, J., Epstein, H.F., and Stryer, L., Segmental Flexibility in an Antibody Molecule, J. Mol. Biol. 51:573-590 (1970).

Veatch, W., and Stryer, L., Effect of Cholesterol on the Rotational Mobility of Diphenylhexatriene in Liposomes: A Nonosecond Fluorescence Anisotropy Study, J. Mol.Biol. 117:1109-1113 (1977).

Munro, I., Pecht, I., and Stryer, L., Subnanosecond Motions of Tryptophan Residues in Proteins, Proc. Nat. Acad. Sci. 76:56-60 (1979).

Further work on the Pasteur probe assays for asymetric carbons led to five papers and two IRL reports (1042 and 1045).

The Atlas CH-4 continued to be used to generate new results in natural products in Prof. Djerassi's laboratory. Six papers were published.

Effort continued on the Slodzian-Castaing system for mass spectrometry microanalyses of solids. An interesting observation was made of a 10-fold preference for negative secondaries to contain an even number of carbon atoms for all observed combinations of primary ions and organic targets. A less pronounced enhancement of odd carbon clumps over even was found for positive secondaries. Observation of odd-even effects has been used as argument in support of the biogenic origin of oil. Our demonstration that such effects can be generated by abiogenic mechanisms would appear to weaken this particular argument. Our results have not been published except in the IRL Status Report (IRL 1050).

The DENDRAL system was fully implemented with respect to acyclic structures. The system was also specified for cyclic structures but implementation was not feasible on the available computers.

Further work was accomplished on computer managed instrumentation. The LINC was used for gas chromatographic curve reading and integration, and hardware developments were completed for direct acquisition of GC data by the computer. Investigations were started in the use of the LINC as an input device to the large time-sharing system (ACME) then being submitted as a proposal to NIH, Division of Research Facilities and Resources.

On January 11, 1966, a proposal was submitted to NASA for \$268,466 to cover an extension of six months to October 1, 1966 and for \$542,223 for twelve months covering the period from October 1, 1966 to October 1, 1967. The fund request for this latter period was revised on August 10, 1966 to \$448,850 and the period changed to cover September 1, 1966 to September 1, 1967. On April 1, 1966, an award was made for the period April 1, 1966 to September 30, 1966. The award covering September 1, 1966 to Sept 1, 1967 was received on December 8, 1966.

The proposal for the first period included a \$25,00 subcontract for a model "Pasteur Probe" based on a proposal dated December 16, 1965 from Electro-Optical Systems, Inc., Pasadena, California (EOS Proposal 65-5116). This development was not carried out and this was, in part, the basis of a reduction in funds requested for the subsequent period.

G. APRIL 1, 1966 - OCTOBER 1, 1966

The work during this period is covered in IRL Status Report 1054.

During this period IRL was relocated in the new facilities provided by NASA grant NSG-(F)-2.

Work on fluorometry continued. In particular a method of

detection and identification of bacteria using a fluorometric aminopeptidase aspay was developed. This used amino acid β -napthylamides (BNA) as substrates.

The instrumentation work on phosphorimetry in collaboration with Dr. Stryer was completed and described in a paper by L. Hundly, T. Coburn, E. Garwin, and L. Stryer "A Nanosecond Fluorimeter" in Review of Scientific Instruments, 38, 488-492, 1967.

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Laboratory demonstrations of the Pasteur Probe Experiment were completed showing detection of as little as 10^{-5} grams of optically active amino acids. Work was initiated on improvements using a Bieman enrichment device seeking an increase in sensitivity of two orders of magnitude.

The Atlas CH-4 Mass Spectrometer in Prof. Djerassi's laboratory produced results yielding six published papers.

A laser input was studied for enabling spatial resolution in the mass spectral analysis of organic solids using a Bendix TOF mass spectrometer. The TOF was tied directly to the LINC computer which was programmed to determine peak positions in the spectra.

Studies were carried out on the melting characteristics of DNA. It was hoped that individual molecular species of DNA could be detected in a heterogeneous sample of sufficiently accurate measurement of the melting curve. The results of a study of the literature made it appear impossible to achieve the objectives of this program.

Dr. Henry R. Hulett and Dr. Danute E. Nitecki joined the Chemistry Group. James Bridges joined the Engineering Group and L. Hundley left the Laboratory.

H. October 1, 1966 - April 1, 1967

The proposed continuation for this period reflected a reduction in scope. It placed greater emphasis on basic science and less on hardware implementation.

IRL Status Report 1056 covers this period.

We carried out investigations jointly with Ames Research Laboratory on fluorometric assays of aminopeptidase activity in microorganisms.

Further studies continued on GLC resolution of diastereoisomers. The technique was used to correlate the absolute configuration of organic compounds. Studies were also carried out on the determination of steric purity of peptides using NMR spectroscopy. The results showed that this is a convenient technique to study racemization in peptide synthesis.

The research in Dr. Djerassi's laboratory using the Atlas CH-4 for the analysis of natual products produced eight papers.

An approach to the analysis of peptides using low resolution mass spectroscopy was investigated. The method involved the incorporation of chlorine into the n-terminal amino acid of the peptide chain.

Research on laser induced vaporization of samples for TOF analysis was continued. The configuration used delivered insufficient focused pulsed areal energy density to meet the research needs. Reinstrumentation to rectify this deficiency was undertaken and yielded a 60-fold increase in energy density.

Work on computer manipulation of chemical hypotheses generated a polished form of DENDRAL running on the PDP-6 LISP system. A report by Georgia Sutherland, Stanford Artificial Intelligence Project Memo No. 49 is included as an appendix to the referenced Status Report.

A special purpose unit termed 270X was built by IBM to provide a high-speed input to the ACME 360/50. interfacing on-line instrumentation.

IRL engaged in cooperative efforts with the Chemistry Department to instrument the AEI MS-9 mass spectrometer in a fast scan, on-line system. Utilizing the 270X and ACME, it was hoped to have an operational computer-controlled data gathering system in operation by November 1967.

Instrumentation interfaces for the EAI Quad 300 Quadrupole mass spectrometer were also developed. Ultimately publication of this system and methodology for computerized operation of quadrupole mass spectrometers played a role in development of commercial products of this kind.

Work was started in this period on a particle or cell separator. This was based on work described by Fulwyler (Science, 150, 910, [1965]) using the resistance principle. This general technique would have applications in problems of both biological and exobiological interest. Consideration of optical techniques using various types of illumination was undertaken emphasizing fluorescence.

Investigations proceeded into the separation of specific fractions of DNA by elution from hydroxyapatite columns. Preliminary experiments showed that with these columns one could distinguish between fully native and partially denatured DNA.

In addition to the papers from Dr. Djerassi's laboratory, four papers were published. Virginia Close joined the Chemistry group.

I. April 1, 1967 - October 1, 1967

The work during this period is covered in IRL Status Report 1061.

Further productive work was carried out on gas chromatography and optical resolution. One phase proceeded with further use of these tools to determine the configuration of asymmetric compounds by gas chromatography of diastereoisomers. Investigations also were carried out into a new approach to

quantitative amino acid analysis using gas chromatography. This involves the conversion of the amino acids to the non-polar isothiocyanate derivatives. Determinations of steric purity of peptides by NMR spectroscopy were carried out.

The analysis of natural products in Prof. Djerassi's laboratory yielded the results reported in 14 papers.

The laser system for solid sample vaporization using the Bendix TOF was completed and the first results on nitrogeneous bases, nucleosides and nucleotides were acquired.

In connection with efforts in computer managed instrumentation an IBM 1800 was acquired and used to interface analog data from various laboratories in the Medical School to the ACME 360. For higher data rates than the 1000 system could support, the IBM 270X Data Adapter was used with four 270Y Experimental terminals.

The computer interface and computer operating system for the EAI Quad 300 quadrupole mass spectrometer was completed and the methods and results published in IRL Report 1062 (November 1, 1967). A similar system was implemented for the Bendix TOF. Progress was made on real-time data acquisition from the MS-9 mass spectrometer in the Chemistry Department to the ACME IBM 360/50.

Further work on computer manipulation of chemical hypotheses was reported in Stanford Artificial Intelligence Memo No. 54, August 2, 1967. This also appeared in "Symposium on Cognition", Carnegie Tech., John Wiley and Sons.

Work continued in the development of the cell separator. Problems with clogging of the orifice or detection structure received much attention. A simulator system was used to evaluate electrical and optical conditions for a high speed flow system which could be used for optical detection of fluorescent cells. These evaluations showed promising results: with a droplet size of 180 micron diameter at 10^{-7} molar concentration, 2 x 10^{8} fluorescein molecules could be detected in 4 x 10^{-4} seconds.

Limited work on attempts to renature specific fractions from thermal chromatography runs on hydroxyapatite columns were carried out.

Efforts were initiated on a joint proposal with Ames in response to the 1973 Voyager experiment opportunitiess based on the Pasteur Probe experiment. This AFO was subsequently suspended.

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Two reports and eleven papers were published including a presentation at the 1966 COSPAR meeting, May 1966, "Relationship of Planetary Quarantine to Biological Search Strategy."

A proposed continuation of the grant for the period September 1, 1967 to August 31, 1968 was submitted September 20, 1967 with a budget of \$478,255. This was revised to \$410,000 on October 27, 1967 and a supplement for that amount was received November 30, 1967.

J. October 1, 1967 - March 31, 1968

The work during this period is covered by IRL Status Report 1076.

Work continued on gas chromatography analysis of amino acids and studies of factors affecting the separation of diastereoisomer compounds by G.L.C.

It was found that menthyl chloroformate is a useful agent for G.L.C. resolution of asymmetric alcohols, amines, amino-acids and hydroxy-acids.

The work in Professor Djerassi's laboratory produced twenty-two papers.

Both laser vaporization and crucible heating were used to generate results using the Bendix TOF. A flexible computer display system was developed for convenient analysis and comparison of the spectral data acquired. Data from twenty-three spectra acquired by one or another or both vaporization methods were studied.

Problems developed in attempting to apply the IBM 270Y units to sophisticated high-speed data acquisition use.

Improvements, both hardware and software, were made to the GLC/MS computer systems. These increased the usefulness of the newly installed Finnigan Instruments Model 1015 quadrupole mass spectrometer.

The generalization of DENDRAL to ring systems has been completed.

The work on cell separation proceeded to the point where there were three instruments capable of carrying out worthwhile biological experiments. These included the volumetric cell separator, a high speed fluorescent cell separator and a third unit, on loan from the Watson Laboratories of IBM, developed under the direction of Dr. L.A. Kamentsky. This has developed into a program involving participation of scientists in several departments working under many different grants.

The following are a few of the medically and biologically significant experiments that were being undertaken.

- 1. Test purified cell populations for cell-cell interactions in the induction, development, and execution of the immune response (in conjunction with Professor Herzenberg and Dr. Weissman).
 - a. Isolation of antigen-processing cells by phagocytosis of fluorogenic substrate.
 - b. Isolation of immunologically competent cells by size.
 - c. Isolation of antibody-forming cells by size following adherence of large antigenic particles.
- 2. Isolation of cells in mitosis by size criteria in order to establish cell lines in vitro which are synchronously cycling. These will be useful to determine the actual cellular and molecular events

which determine the differential sensitivity of cells to radiation and certain drugs as a function of their place in the cell cycle (in confunction with Professor George Hahn, Department of Radiology).

- 3. Detection and isolation of cancer cells in the blood stream (metastases) in order to determine the type of cancer therapy most appropriate for the patient (in conjunction with Professor H.S. Kaplan, Executive Head of Radiology and Radiotherapy and Professor R. Kallman, Radiology Department).
- 4. Isolation and testing of cell types in Hodgkins Disease, (a cancer of the lymph node system) in order to determine:
 - a. the malignant cell, its biochemistry and radiosensitivity.
 - b. the cell type (in these patients) responsible for widespread immunological deficiency, and how this deficiency is maintained (in conjunction with Professor H.S. Kaplan).
- 5. Isolation and testing of the cell type in the bone marrow theoretically designated as the "stem" cell, which is responsible for redevelopment of normal blood chil types following irradiation (in conjuntion with Dr. Weissman). (NASA and the Air Force already have supported projects and symposia aimed at answering just this question, for the purpose of possible treatment of radiation exposure in space.)

A unique method of making observations on the jet stream after it has left the jet forming orifice was developed. This greatly improved the functioning of the system.

Work on the Kamentsky unit was directed toward studies of fluorochromasia, the appearance of fluorescein in cells because of enzymatic hydrolysis of non-fluorescent fluorescein diacetate (FDA) to give fluorescein and its retention within the cell.

In addition to the papers from Dr. Djerassi's laboratory, three reports and seven papers were published.

K. April 1, 1968 - September 30, 1968

IRL Technical Report 1082 is a status report covering this period.

Work on gas chromatography of amino acids was noted by two accomplishments, both of which were published. The process of obtaining volatile derivatives of amino acids was considerably accelerated by a method of using the injector port of the G.L.C. as a chemical reactor. Excellent yields, in some cases chromatographically pure, were obtained by an application of transesterification procedure to the cleavage of peptide bonds from the resin support using methanol or ethanol.

Further work continued on sequence analysis of peptides by mass spectrometry. In incorporating chlorine into the N-terminal amino acid the observation was made that the presence of phenylalanine in the peptide results in the evaporation of a chlorine atom in the aromatic ring of the phenylalanine.

Professor Djerassi's laboratory contributed five papers to the literature.

Additional hardware and software refinements were made of the Bendix TOF system for mass spectral analysis of organic solids. The instrument carried out test analyses using mice lymphocytes and human red blood and fibroblast cells.

Continued development took place of the several computerized mass spectrometry systems.

In the cell separation program work continued on the application of the IBM rapid cell spectrophotometer to fluorochromasia. The technique appears to be adaptable to automation of a fluorochromatic cytoxity assay developed by Bodmer, Tripp and Bodmer. It was being used to investigate heparin in mast cells.

Preliminary biological experiments were conducted to validate the completed design of the volumetric cell separator. The high speed fluorescent cell separator results were encouraging in that the intrinsic capability of the system was validated but poor reproducibility indicated the need for improvement in some defined areas.

Fourteen reports, papers and publications were produced, in addition to those of Professor Djerassi's laboratory.

A request for \$395,000 in funds for the continuation of this grant for one year was submitted July 2, 1968. This was resubmitted as a request for \$340,000 for a ten month period on July 30th. This was awarded on October 22, 1968 for the period September 1, 1968 to July 1, 1969. This continuation proposal was based on a plan to seek additional support for the laboratory from other sources or to reduce the staff if necessary.

On September 30, 1968, we were informed that effective November 1, 1968 the grant was changed from NSG-81 to NGR 05-020-004.

L. October 1, 1968 - July 1, 1969

The work during this period is covered by two status reports, IRL Report Numbers 1087 and 1091 covering the periods October 1, 1968 to December 31, 1968 and January 1, 1969 to March 31, 1969 respectively, and a summary report, IRL Report 1092, covering July 1, 1968 to July 1, 1969.

The new methods developed for fast GLC amino acid analysis were successfully used for the biochemical screening of blood samples: for high

phenylalanine in the detection of phenylketonuria and for the quantitative determination of radiobiological agents in biological materials.

The low resolution mass spectrometric method of sequence analysis of peptides, using the approach of incorporating an enamine-ketone moiety into the N-terminal amino acid of the peptide chain, succeeded in giving the correct sequence in model systems containing up to 10 amino acids.

The laser/mass spectrometer solids microanalysis system was developed to routine operational capability. The spatial resolution is 50 microns and the sensitivity is approximately 1 x 10^{-9} gram.

The system was used in connection with the Lunar Sample analysis program of G. Hodgson in the application to a number of porphyrin compounds. It was also used by B. Halpern in analysing small amounts of material resulting from the chemical extraction of samples separated by GLC on silica gel.

Work with the Pathology Department to examine differential histamine content of normal and lesion human tissue was unsuccessful in finding characteristics distinctive of abnormality.

Eight publications resulted from the work in Professor Djerassi's laboratory during this period.

Investigations were carried out to examine the structure of extraterrestrial porphyrin on the basis of molecular size, structural configuration, and association with amino acids. The application of magnetic circular dichroism was explored as a sensitive spectroscopic technique for the detection of a biologically important class of compounds, the chlorophyll-like porphyrin which may be present in a lunar sample.

Modifications to increase sensitivity of the magnetic circular dichrometer were carried out as well as interfacing to the ACME 360-50 computer in preparation for searching for very small quantities in lunar samples.

The development of computer aided instumentation, particularly GLC/MS, was carried to the point where it not only related closely to possible interests of the Viking Mission but also attracted a great deal of interest for laboratory application.

The fluorescent cell sorter test of cell wall integrity (fluorchromasia) appeared sufficiently useful in immunological assays that a contract was received from NIH to explore further automation of the test.

Some experiments were carried out to investigate the feasibility of supplementary data processing using optical methods for analysis of Mariner Mars 1971 orbital images.

A separate task was carried out to develop a variable time-lapse videoscintiscope for the Division of Nuclear Medicine. This was a particularly successful attempt to transfer technology resulting in important

clinical improvements in the use of scintillation cameras for cardiac and pulmonary disease diagnosis.

The work during this period resulted in twenty papers and publications in addition to those of Dr. Djerassi's laboratory.

On April 9, 1969, the grant was extended to August 31, 1969 within the funds previously obligated.

A request for \$342.899 in funds for continuation of this grant was submitted together with a request for \$27,900 in funds for capital equipment. The capital equipment request was not granted. A grant for \$340,000 additional funds covering the period September 1, 1969 to August 31, 1970 was awarded July 29, 1969.

M. July 1, 1969 - July 1, 1970

The work during this period is covered by a status report, IRL Report 1105, covering the period July 1, 1969 to January 1, 1970, and a summary report, IRL Report 1110, covering the complete period.

The techniques developed on GLC Separation of D and L antipodes were applied successfully to determine the absolute configuration of the alloisoleucine present in the serum of patients suffering from "Maple Syrup" disease. Collaboration continued with Ames personnel in the construction of a working model of a hydrolyser-desalter derivatizer unit that could be the basis of a Viking Mission instrument.

A prototype of a partially automated sample injection system for glc determination of blood phenylalanines. This should be of use in the routine screening of newborns for phenylketonuria.

Studies continued in the reaction of chlorine with DNA. The formation of chloramine derivatives of nucleosides being a possible toxic hazard.

Under a separate contract (NAS 9-9439) we examined samples of Apollo 11 and 12 for "porphyrin-like" pigments. Abundances of 10^{-4} microgram/gram were found but other data suggests that it was introduced by exhaust products from a lunar descent engine.

Nine papers were produced by Dr. Djerassi's laboratory.

Further work on DENDRAL extended its application to other classes of organic molecules such as alephatic ethers. Work was started on more advanced phases such as "Meta-Dendral" which deduces chemical reaction rules from the MS data and "Synthetic Dendral" to design and test reaction sequences for synthesis.

The computer aided research instrumentation efforts continued. A commercial version of the GLC-MS computer control interface was produced by Systems Industries, Sunnyvale, California.

Most of the support for cell separation research was now coming from non-NASA sources (NIH grant GM 17367 and NIH contract 69 2064). An improved system using sheath flow enclosing the stream of fluid containing the cells in a larger stream of inert liquid was built and tested. This gave improved operation for both fluorescent cell sorting and high-speed volumetric sorting.

Development of optical data processing continued and demonstrated its value in image enhancement and noise removal for images from the 1964 Mariner fly-by.

During this period Professor Lederberg and Dr. Levinthal were directly involved in Mariner Mars 1971 and Viking 1975 mission activities.

Professor Lederberg was a principal investigator on the MM '71 Television Team and Chairman of the Exobiology Discipline Group of that team. Dr. Levinthal was co-investigator on the team and a member of the Exobiology Discipline Group. He served on the Hardware Task Group and was chairman of the Data Processing Task Group and a member of the Mission Operations Planning Group.

Professor Lederberg was a consultant to the Biology Instrument Team and an investigator on the Biology Science Team for the Viking '75 mission. Dr. Levinthal served on the Imaging Instrument Team and as an investigator on the Lander Imaging Science Team.

Dr. Halpern was a co-investigator on one of the Lunar Sample Analysis Teams.

Drs. Lederberg, Levinthal and Halpern were supported in these activities by other members of the Instrumentation Research Laboratory.

Some theoretical and experimental studies, by Dr. Liebes, were done on a "quasi-microscope" that could be added to the proposed Viking lander camera for a 40-fold magnification.

Work was carried out in conjunction with Lynn Quam of Professor John McCarthy's Artificial Intelligence Laboratory for analysis of Mariner Mars 1971 orbiter photography. This work was fundamental to studies of variable surface features.

Thirty-four papers and publications resulted from the work of this period.

A request for \$290,000 in additional funds was made on July 23, 1970. This was awarded on September 17, 1970.

N. July 1, 1970 - January 1, 1971

The research during this period is covered in IRL Status Report 1123.

Research was initiated on chemical methods for the analysis of the basic and acidic constituents of urine. This required redesign of the GC/MS interface to improve sensitivity. Chlorination studies of bases present in DNA were continued. A new quantitative spectrophotometric method for the detection of cyclohexyl amine was developed. Cyclohexyl amine is known to be toxic.

The work in Dr. Djerassi's laboratory produced four papers.

Many mass spectra were taken to gather data for the DENDRAL program. This included steroids which added new dimensions to the computer analysis problem.

Work was completed or a "smart" terminal project (called "Hi Q") carried out in collaboration with Professor Melvin Schwartz of the Physics Department. This provides a local interface for a mini-computer (such as a PDP-11) for dedicated real-time instrumentation. The mini-computer is, in turn, connected to the larger (ACME) time-shared facility.

Work continued on cell separation techniques now mostly supported by NIH grants and contracts.

A system for analysis of Mariner Mars 1971 orbiter photographs suitable for "production" use during the ninety days of the mission was under development. This system included a modified Huffman coding scheme for data compression, a computer based "atlas" of Mars and methods for precision geometric and photometric registration.

During this period, Dr. Halpern left the laboratory to return to Australia. His responsibilities were assumed by Dr. Alan Duffield.

On June 29, 1971, a request was made for \$240,000 to continue the grant for the period September 1, 1971 to August 31, 1972. This was awarded September 1, 1971.

0. January 1, 1971 - December 31, 1971

IRL Status Report 1128 covers the work during this period. It also contains a general review of the DENDRAL Research Project. Because of the richness of the problem environment provided, and the success of DENDRAL in solving real problems, the effort has become the focus of much attention and comment in the Artificial Intelligence community. Considerable progress was made in the structural analysis of estrogenic steroids by Heuristic Dendral and toward Meta-Dendral goals of theory formation.

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In the area of computer aided research instrumentation, capabilities were developed for remote micro-computer programming, combining the advantages of a stand-alone instrumentation system with the power of a large machine and high level language processor. The new MAT 711 was brought on-line to the PDP-11.

In the studies of urine constituents, following chemical derivatization of the acidic fraction, over 80 individual peaks were observed on the GC. We were unsuccessful in isolating the catecholamines from the basic fraction but did develop a derivatization procedure for their GC/MS identification.

The method of analysis of phenylalanine in serum was completely automated allowing one hundred samples per day to be screened using a four-column GC.

A method was developed for determining amino acid constituents of soil quantitatively using deuterated amino acids as internal standards.

Further work continued on the chlorination of DNA bases and the mass spectrometry of organic compounds.

Dr. Djerassi's work on MS of natural products produced four papers.

The picture processing developments for Mariner Mars 1971 orbiter photography were completed and used to study variable features. In addition specially enhanced images of Phobos were produced.

On April 22, 1971, a request was made for \$50,029 in supplemental funds to contribute to the purchase and use of a Varian-Atlas mass spectrometer. This was granted on May 21, 1971.

During this period Drs. Lederberg, Levinthal and Liebes participated extensively in the Viking Lander investigations. Specific work was done using the facilities of the Stanford Al laboratory on developing the tools for extracting near-field range information from lander camera stereo pairs.

Eleven papers and reports were produced during this period, in addition to those of Dr. Djerassi's laboratory.

A request for \$180,000 in additional funds was made September 21, 1972 and granted October 24, 1972 for the period September 1, 1972 to August 31, 1973.

P. January 1, 1972 - December 31, 1972

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This period is covered by IRL Status Report 1158.

The study of the action of aqueous hypochlorous acid under physiological conditions on bases present in DNA continued. The study was extended to nucleosides, cytidine and deoxycytidine and the nucleotide cytidine- 5^1 -monophosphate. Two papers describing this work were published.

Using the methods of mass fragmentography and the quadrupole-mass spectrometer-computer system, it was possible to simultaneously quantitate up to ten of the amino acids present in soil. This quantitative technique was extended to the measurement of phenylalanine in plasma. Both the above results were published.

The types of rearrangements occurring in mass spectral fragmentation were studied for promazine and its sulphoxide. These phenothiazines represent an important class of drugs used as tranquilizers.

Work progressed on the study of organic chemical constituents of the urine of premature babies. A specific study related to late metabolic acidosis.

Dr. Djerassi's laboratory work on natural products produced seven papers.

The status report included Chapter 7 of Biochemical Application of Mass Spectrometry, Edited by G. Waller (Wiley and Son , 1972) entitled "Use of A Computer to Identify Unknown Compounds: The Automation of Scientific Inference" by Joshua Lederberg. This summarized the progress in the DENDRAL Artificial Intelligence Project to date.

Using the MAT-711 high resolution mass spectrometer, we made progress toward a reliable, automated data acquisition and reduction system for scanned low and high resolution spectra. The system includes extensive automation of operations, sophisticated automatic instrument calibration and data reduction programs and a new algorithm for separation of unresolved peaks giving a factor of three improvement. Using a unique disk oriented matrix transposition algorithm the GC spectra are converted to a "mass fragmentogram" form of the data giving much higher resolution in localizing GC effluent constituents.

The cell separation group completed a multichannel cell separator which simultaneously measures fluorescence and scattering cross-section of each cell.

The Mariner Mars 1971 image processing work, done in collaboration with the Stanford Artificial Intelligence Laboratory resulted in 500 image differencing operations on MM '72 photos. In addition 31 special enhancements of Phobos and Demos were executed.

Drs. Levinthal and Liebes, as members of the Lander Science Team

assumed reponsibility for the Science Operation Requirements Document (SORD), the Software Functional Descriptions (SFDs) and the more detailed Software Requirements Documents (SRDs).

Q. January 1, 1973 - July 31, 1973

The activities under this grant for this period are summarized in the proposed continuation for the period September 1, 1973 to August 31, 1974 submitted August 10, 1973. This proposal request for \$150,000 was granted on October 16, 1973.

The techniques of quadrupole mass fragmentography were further developed achieving quantification of amino acids below the nanogram level. The advantages achieved over older methods of fragmentography using sector mass spectrometers are the large mass range that can be scanned, simplicity of computer interface for data acquisition and ability to monitor a large number of ions. The system is now "stand-alone" using a PDP-11/20 mini-computer.

We succeeded in quantification of β -aminoisobutyric acid in urine in patients suffering from leukemia meeting a need for a reliable method of measuring this compound in urinary excretions. We extended mass fragmentography to the quantification of ethanol in blood and urine using deuterated ethanol as a standard.

We commenced a collaborative investigation with Dr. James G. Lawless of NASA-Ames Research Center, using these techniques on the amino acid composition of the Murchison meteorite. This technique is potentially suitable for planetary investigations.

Preliminary investigations were carried out on extraction techniques and the absorption and retention of selected amino acids. Preliminary results, using GC/MS of the constituents of an extract of the Murchison meteorite indicated the presence of a series of aliphatic dicarboxylic acids.

We received a grant of \$60,000 from NASA-Johnson Space Flight Center (Grant NASA 05-020-632). This was under the Office of Life Science, NASA, Biomedical Research Division, Dr. Sherman Vinograd, Director. The grant, entitled "Analytical Methodolgy for Biochemical Monitoring" was initiated May 12, 1973, in response to a proposal submitted April 13, 1973. This was a revision of a proposal for \$160,000 previously submitted in July of 1972. The aim of this research was related to our studies under this grant. It was to perfect methods for the automatic monitoring of astronauts' health and nutritional status. The baseline studies were carried out on premature infants since they, like astronauts, are kept on a rigid diet. This study was carried out in collaboration with the Pediatrics Department and involved 100 urine samples from 11 premature infants.

The transition of the ACME computing facility from an NIH subsidized IBM 360/150 to a fee-for-service IBM 370/158 necessitated changes

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in the high and low resolution GC/MS data systems. The initial stages of transfer to a stand-alone PDP-11/20 system were completed.

Further improvements in the data analysis programs supporting the GC/MS systems were accomplished.

Participation of Professor Lederberg and Drs. Levinthal and Liebes continued in the Viking mission.

The new proposal allocated about one-third of the funds to a new subproject applying recent findings in the artificial incorporation of synthetic DNA sequences to issues of molecular evolution.

R. August 1, 1973 - July 31, 1974

The status report for this period was incorporated in the request for continued support submitted August 14, 1974. This request for \$150,000 for the period September 1, 1974 to August 31, 1975 was granted September 25, 1974. This support included \$40,000 towards the purchase of an electron microscope.

We continued our collaboration with Dr. James G. Lawless of NASA Ames Research Center. The successful use of quadrupole mass fragmentography led to publishing the quantities of amino acids present in the Murchison meteorite. This included leucine and isoleucine which had not previously been detected in extracts from this meteorite. The remaining six protein amino acids were detected in concentrations in good agreement with other published results.

The degree of racemization, accompanying the powdered meteorite catalyzed hydrogen-deuterium exchange in an aqueous solution of deuterated amino acids, or a deuterium oxide solution of protein amino acids, was also measured.

Another published result was the isolation and identification of aliphatic dicarboxylic acids in the Murchison meteorite. Methyl succinic acid was estimated to be present at a concentration of 24 micrograms per gram of meteorite, an order of magnitude greater than the amino acids.

This result motivated the successful search for dicarboxylic acids in spark discharge experiments.

Work was carried out on using ^{13}C labeled amino acids as internal standards instead of deuterated amino acids. This removes ambiguities due to efficiencies of solvent extraction procedures and gives a more accurate indication of amino acid content of ten meteorite/soil samples.

In connection with the Applications Laboratory of Finnigan Instruments Corporation, we investigated the use of chemical ionization — mass fragmentography for the subnanogram detection of metabolites present in urine.

Two new significant improvements were incorporated into the analytical system for the recording of mass fragmentographic data. We introduced software modification to allow an extended dynamic range to be utilized in the recording of mass spectra. With a computerized approach to encoding we were able to use standard 12 bit A/D converters. The sampling time is adjusted so that no reading exceeds the 12 bit range and after each reading the A/D is set to zero to measure the next subinterval. The incremental value read is accumulated with the computer.

The second improvement repetitively samples each of the masses to be measured only during the time of their elution. This allows each pair of ions (the amino acid derivative and its deuterated analog) to be monitored for longer periods of time, significantly improving sensitivity.

Investigations were carried out on improving the computerized search routines through a library of reference mass spectra. This required new techniques for the removal of background contributions due to column and system bleed.

The library of mass spectra relevant to our chemical techniques of derivatization were sent to Dr. Sanford Markey, Denver, Colorado, where they were compiled with other biologically relevant mass spectra from other laboratories.

We investigated, at the instigation of Dr. Steven Heller, EPA, a method for the routine transcription of existing bar graph data into digital format using an electronically sensitized tablet. This will allow incorporating 8000 mass spectra from Professor Djerassi's laboratory into the EPA data library.

A temporary solution to the problem of computerized high resolution mass spectrometry using a PDP-11/20 IBM-1800 interface to the 360/158 was generally unsuccessful. Efforts were undertaken to recode the software into FORTRAN anticipating the acquisition of PDP-11/45 systems to solve the problem.

Participation of Professor Lederberg and Drs. Levinthal and Liebes in the Viking mission significantly increased during this period.

S. August 1, 1974 - July 31, 1975

The status report for this period was incorporated in the request for continued support submitted July 30, 1975. This request for \$110,000 for the period September 1, 1975 to August 31, 1976 was granted September 23, 1975.

This NASA grant provided the underpinnings for the health oriented research now dominating the activities of the IRL. This work is now largely supported by other grants. These include NIH 5R24 RR 0612; NCI Contract NO1-CB-43909; NASA NGR-05-020-632; and NIH 1P01 GM 20832-01A1.

The emphasis of the laboratory work supported by this grant is now on molecular evolution. A general description of this work is given in Attachment 35 which was prepared as a Research and Technology Resume in response to Research and Technology Objective and Plan (RTOP) 192-55-64 Biological Adaption. During this period it was demonstrated how the chromosome of Bacilus subtilis can be segmented with Eco-R1 nuclease into about 200 pieces of average length of about 10,000 nucleotide pairs. These segments can then be fractionated with agarose gel electrophoresis giving DNA bands which are purified to the extent of about 30% absolute purity. (R.M. Harris-Warrick, Y. Elkana, S.D. Ehrlich and J. Lederberg; "Electrophoretic Separation of Bacillus Subtilis Genes" PNAS Vol 72, No. 6, pp. 2207-2211, June 1975.)

These segments have been ligated to the small plasmid psc 101 giving rise to a wide range of clones used to map the genome of the source bacterium. We have not been able to verify the expression in E. Coli of genetic information derived from B. subtilis. Radiolabelled RNA, transcribed from a DNA clone, has been used to stain an electrophoretogram of the B. subtilis DNA and to show that each DNA clone contains one or more specific segments.

We have also studied the complex segments that are left by incomplete digestion of the source DNA and showed how this could be used to map a sequence in relation to neighboring segments.

We encountered greater methodological problems than anticipated in the original purpose of introducing artificial synthetic monotonous sequences of known composition into B.subtilis and into plasmids for further studies of molecular evolution but made progress with the underlying issues.

The difficulties in computerized interpretation of GC/MS data introduced by componenets unresolved by the GC and by interference from GC column "bleed" were alleviated by the spectrum "CLEANUP" program. This takes as input an entire GC/MS run (600-700 spectra) and returns as output the set of mass spectra of detected components (30-50 for a typical run of a chemical fraction of human urine).

We examined the Murchison meteorite for sterols. Within the limits of detection of the GC/MS system, we were unable to detect any sterols. The only compound identified was the plasticizer, tri-n-butylphosphate, whose origin is unknown.

Initial work on ¹³C labeled amino acids used a sample of crude ¹³C protein from anacystis nidulaus supplied by Dr. Donald C. Ott. Group Leader of the Organic and Biochemical Synthesis Group, University of California, Los Alamos Scientific Laboratory. This enabled selection of the appropriate ions for monitoring by mass fragmentography. A commercial ¹³C labeled amino acid mixture from Merck was ordered to be used for further assays.

The necessary computer routines for quantitative analysis using the compounds was completed.

The PDP 11/45 system was delivered and interfaced to the Varian MAT 711 high resolution spectrometer via the existing PDP 11/20. The necessary software developments were completed and included several improvements.

The library of 3000 mass spectra of biologically relevant compounds was received and its use initiated. The encoding of 2500 of the 6000 mass spectra of the collection of Professor Djerassi was completed for inclusion in this library.

The development of improved computerized library search routines continued. Some of this work was conducted with the collaboration of Dr. S. Grotch of JPL. Progamming efforts led to the development of general solutions to the problem of background removal with concomitant deconvolution of overlapping GC peaks. These programs became operational.

A non-technical memo (Attachment 36) was supplied to Dr. Richard Young of the NASA Program Office. This, together with an article published in the Stanford M.D. (Fall '74) "Are There Microbes on Mars?" (Attachment 37), attempts to describe in lay terms some of the intents and activities of our laboratory.

During this period Professor Djerassi's laboratory produced seven papers on the structural analysis by MS of natural products.

Fourteen other papers and reports were authored by members of the IRL.

The participation in the Viking mission further intensified during this period.

T. August 1, 1975 - July 31, 1976

The status report for this period was included in the propsed continuation for this grant, submitted August 25, 1976. This propsal requested \$137,500 for the fifteen month period from September 1, 1976 to November 30, 1977. These funds were granted November 1, 1976.

The problem of expression of successfully transferred DNA segments was discussed in the previous year's report summary. This problem was solved and the results published (Ehrlich, Bursztyn-Pettegrew, Stroynowsky and Lederberg, PNAS, Vol. 73, No. 11, pp. 4145-49, November 1976). This describes the expression of the thymidylate synthetase glu2 derived from B. subtilis phage Phi-3-T. Similar results were achieved with respect to a certain number of ordinary chromosomal markers of B. subtilis. A puzzling result is the exclusion, in the reinsertion into B.subtilis of the expressed segments by the ususal transformation procedure, of most or all of the plasmid DNA of E. coli prevenience and to accept only that originally derived from B. subtilis.

The crucial success is the identification of effective promoter regions from the Phi-3-T phage capable of initiating the expression of the attached DNA. This allows progress toward the goal of incorporation of specified synthetic sequences.

Further improvements were made in the "CLEANUP" program for automatic detection of components in a Gas Chromatograph/Low Resolution Mass Spectrometer (GC/LRMS) run. These give more reliable component detection and add curve-fitting procedures which aid in resolution of overlapping, multi-component GC elutants. This has improved library matching procedures.

We completed a first version of a program that extended the procedures for preliminary analysis of GC/LRMS data by (1) calculation of relative retention indexes for each component; and (2) by determination of semi-quantitative values for relative concentration of components.

We started design and implementation on combining a number of experimental GC/LRMS results to accumulate a record of past observations. This is to be used to utilize an historical record yielding a subsequent basis for comparison with new data.

Based on GC/LRMS and GC/HRMS experiments, together with the CONGEN program (CONGEN was developed as part of the DENDRAL project - Ref. 3 [page 42 of proposal]) for computer-assisted structure, we have determined the structures of these isomeric compounds in the urine of a patient with symptoms of mental retardation. The compounds are conjugates of o, m and p-methylbenzoic acids with glycine with relative concentration corresponding to those in commercial preparations of xyline to which the patient has been extensively exposed.

During this period. Dr. Levinthal took on responsiblities as Deputy Leader of the Viking Lander Camera Team.

U. August 1, 1976 - December 1977

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The status report for this period was included in the proposed continuation for the grant submitted August 11, 1977. This proposal requested \$110,000. Grant funds of \$100,000 were received January 9, 1978 covering the period December 1, 1977 to December 1, 1978.

To reflect the change in emphasis the title of the grant was changed to "Molecular Evolution in Primitive and Simple Biochemical Systems" on September 9, 1977.

The Research and Technology Resume (Attachment 38) supplied June 23, 1977, in response to RTOP 192-55-67 describes this general effort.

The genetic studies reported on the prevalence of mechanisms for promiscuous mixing of genes among bacteria believed to be quite separate in an evolutionary sense, e.g. Bacillus and Staphylococcus. This unexpected finding

may be an important contribution to the theme of the origin and evolution of life.

Two papers report on this work. They are S.D. Ehrlich, "Replication and expression of plasmids from Staphylococcus aureus in Bacillus subtilis," PNAS, Vol. 74, No. 4, pp. 1680-1682, April 1977, and Ning-Yen W. Chi, 3.D. Ehrlich and Joshua Lederberg, "Functional Expression of Two Bacillus subtilis Chromosomal Genes in Escherichia Coli, J. Bacteriology 13 (2) 816-821, 1977.

The work on analytical methodology of GC/MS is summarized in a series of papers. The first paper is Dennis H. Smith, Michael Achenbach, William J. Yeager, Patricia Anderson, William L. Fitch, and Thomas C. Rindfleisch: "An Approch to Quantitative Comparison of Combined Gas Chromatographic/Mass Spectrometric Profiles of Complex Mixture." This embodies the plans worked on in previous periods.

GC/MS data are processed to locate points of component elution and to obtain mass spectra free from background and contributions from overlapping spectra. Subsequently, spectra of added hydrocarbons are located and their elution times utilized to determine relative retention indexes (RRI's). Then spectra of internal standards are located and the concentration (based on peak areas) of each component relative to the standard is determined. These data, the collection of "clean" mass spectra, RRI's and relative concentrations, constitute the GC/MS profile for a mixture. We developed methods for collecting these profiles into libraries to obtain an historical record of past observations. We also developed the necessary programs for comparing new data to an historical library. The results of the comparison quickly indicate which components are present in abnormal amounts. The collection of programs available in our laboratories constitutes a powerful tool for analyzing and comparing complex mixtures of any organic compounds which can be suitably derivatized for analysis by GC/MS. We used the historical library approach to validate our analytical procedures for isolation of organic material in human body fluids. We used the approach to establish baselines on organic constituents of both urine and amniotic fluid.

Two additional papers (Smith and Carhart, "Structure Elucidation Based on Computer Analysis of High and Low Resolution Mass Spectral Data", 1977, and Carhart, et al. "Computer Assistance for the Structural Chemist", 1977,) discuss approaches to interpretation of mass spectral and other chemical data in terms of molecular structure. These studies, carried out together with our collaborators in the SUMEX and DENDRAL projects, have used actual unknowns discovered during the course of our GC/MS analyses of mixtures. GC/LRMS and GC/HRMS experiments provided mass spectral data on unknowns. Subsequently, structural candidates for these unknowns were proposed based on computer-assisted analysis of the mass spectral data together with other chemical information. The techniques discussed in these papers are also quite general, and like the historical library appoach, can be used in the study of diverse chemical problems, including those outlined in subsequent sections.

We made considerable progress in improving and extending our library of mass spectral data. We added new spectra to the library by running spectra of standard compounds and processing the spectral data with the

CLEANUP program. We improved the quality of existing spectra using the historical library approach outlined above ("HISLIB", Smith, et al., 1977). HISLIB averages spectra of the same compound observed in several GC/MS runs. Thus, statistical variations in ion abundances are reduced as additional spectra are averaged. The resulting spectrum is frequently of much higher quality than spectra in existing libraries. We implemented a mechanism for adding averaged spectra to or replacing spectra in our primary library. This provides a mechanism for gradual improvement of our libraries with time. In addition, relative retention indexes are included with the spectra now, enabling our improvement in the certainty with which subsequent spectra are matched to the primary library.

This period encompassed a period of peak Viking activity for Professor Lederberg and Drs. Levinthal and Liebes.

V. December 1, 1977 to end of grant, May 30, 1980

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In November of 1977, the possibility arose that Professor Lederberg might either be taking an extended leave or moving to another position at another location. In early 1978, his appointment as President, Rockefeller University, was announced. A great deal of the effort during this period was devoted to completing work underway and facilitating the orderly transfer of equipment and programs.

Two extensions of the grant period, without additional funds, were authorized. The first on October 2, 1978 to November 30, 1979 and the second on August 31, 1979 to May 30, 1980. These extensions also changed the principal investigator to Elliott Levinthal; Profesor Lederberg having left for Rockefeller University in September of 1978.

A great deal of Dr. Levinthal's activities during this period continued to be associated with the continuation of the Viking project and with publications of the results. He produced a stereo movie "Mars in 3-D" which presented the lander imagery in three dimensions.

The attached bibliography lists publications that have directly or indirectly resulted from the work under this grant. It is presented in three sections. Section I gives those papers, reports and publications that originated in the Instrumentation Research Laboratory (IRL). Section II lists papers from Dr. Djerassi's laboratory's work on natural products. The facilities of this laboratory were supported by IRL. Section III contains those papers from Dr. Lederberg's laboratory, supported by NASA, related to studies in molecular evolution.

Appendix A gives a summary of the NASA funds awarded during the period April 1, 1960 to May 30, 1980.

APPENDIX A
Summery of NASA Funds Awarded

	PERIOD From To		AMOUNT	
GRANT	4/1/60	3/31/61	\$ 37,605	
Supplements				
1.	4/1/61	3/31/64	380,640	
2.	4/1/63	3/31/64	132,000	
3.	4/1/54	3/31/65	485,120	
4.	2/1/64	1/31/65	62,984	(Mechanical Eng. Subtask)
5.	4/1/65	3/31/66	349.899	(includes \$52,469 for Mechanical Eng. Subtask)
6.	4/1/66	9/30/66	268,460	
7.	9/1/66	8/31/67	448.850	
8.	9/1/67	8/31/68	410.000	
9.	9/1/68	8/31/69	340,000	
10.	9/1/69	8/31/70	340,000	
11.	9/1/70	8/31/71	290,000	
12.	5/21/71		50,029	(Varian-Atlas Mass Spectrometer)
13.	9/1/71	8/31/72	240,000	
14.	9/1/72	8/31/73	180,000	
15.	9/1/73	8/31/74	150,000	
16.	9/1/74	8/31/75	150,000	
17.	9/1/75	8/31/76	110,000	
18.	9/1/76	11/30/77	137,500	
19.	12/1/77	12/1/78	100,000	
Total funds awarded during period April 1, 1960 to May 30, 1980 \$4,				

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Section III

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March 4, 1959

Dr. Robert Jastrow NASA Washington 25 D.C.

Dear Bob:

I am sure you must be preoccupied right now with COSPAR doings, and will ask you to look this over only at your convenience. I have been rather frantic to try to get some of this stuff out before CETEX, so as to avoid athe necessity of going myself. Since the Russians apparametly are not coming, this doesn't seem terribly urgent. With a couple of obvious exceptions, restated in the attached, the CETEX report seems a fairly sound base, and Hughes has a constructive viewpoint.

I would like to raise the question with you of getting an NASA contract to do some spadework, mainly consultation, on the generalities of biological probes. Our phone and travel bills are bound to mount, and also I would like to get some help from a young astronomer at Yerkes--Madison (name Carl Sagan) who is well informed and deeply interested in planetary biology. In fact, I would like to bring up the specific project of bringing out a solar system handbook, in fairly critical fashion, for the benefit and exceitement of the many biologists who should be contributing to the problem.

No word yet from Newell, possible for same % COSPAR reasons. But I may meet him +/cr Glennan at Caltech nx on the 21'.

Yours,

PS: Please do note one change of emphasis.

In my first minute, I advocated minimizing but not necessarily excliding viable contamination from moonshots, mainly for lack of confidence in doing this well. But I now think decontamination can be done, by gaseous fumigation, not by perfunctory swabs with alanks absolute alcohol. I heard one last minute concession for Pioneer IV: they used 70 pc. alcohol, which is at least a more effective germicide. Also the moonshots will play the same developmental role as they do in the engineering for planetary probes—they will tell what your empirical problems (viz. of decontamination) actually are in the field.

Ref. DSA/GFS

March 25, 1959

Dr. G. F. Schilling, Chief Astronomy and Astrophysics Programs National Aeronautics and Space Administration 1520 H Street Northwest Washington 25, D. C.

Dear Dr. Schilling:

The handbook is only part of the program we have in mind. I am enclosing a somewhat more complete statement that may clarify the grounds of our request. This is, of course, a preliminary formulation; formal proposals will be routed through University channels and will include, for example, the usual allowance for indirect costs on the part of the University.

What I would like to stress is the need for some expedition, especially with regard to parts A and B, to assure the continuity of this series of studies.

Copies of the enclosures have been sent to Dr. Odishaw at the Space Science Board. From talking with Dr. Rossi, I gather that there is some uncertainty as to just how far towards operational work NAS should go but I am hopeful that between NASA and NAS the appropriate means can be found to continue our work. Whatever proceedure will result in the least diversion of my own time from the scientific issues themselves would be most welcome, as I need hardly point out.

Yours sincerely,

Joshua Lederberg Professor of Genetics

Enclosure

Preliminary studies on Planetary Biology

At the request of Prof. Bruno Rossi, acting for the NAS Space Science Board. Prof. Joshua Lederberg convened a group of biologists at Wast Coast universities to review some problems of policy in the space research program (particularly biological contamination), to suggest some tangible experimental approaches to the detection of life on other planets, and to stimulate broader interest on the part of biological scientists generally so as to evoke further proposals for experimentation. This groupphas met on two occasions so far: February 21, 1959 (Stanford) and March 21, 1959 (Jet Propulsion Labs., Pasadena); a next meeting is scheduled for May 3, 1959 (Hopkins Marina Station of Stanford University, Pacific Grove). In addition to the members listed below, we have had representatives from the Stanford Research Institute, and from JPL and NASA (A. Hibbs and R. Davies). We have reported to the NAS through Rossi and Odishaw at the Washington office, and to NASA via Hibbs and Davies, and also by letter to Jastrow (on the lunar exploration working group at NASA headquarters). We have also reported to COSPAR (CETEX) by correspondence with Hughes and with Peter Alexander. A jargon, self-indicated name for our group has been "WESTEX". Its university members have been: (1) signifies one mtg. attended.

U. of Cali	fornia (Berkeley):	U. of Cal. (Davis):					
Calvin'	Chemistry	Harr	Bacteriology				
Mazia(1) Stanier(1)	Zoology Bacteriology		(La Jolla):				
Stent Weaver	Virology Astronomy	Urey (1)	Chemistry n				

The composition of the group therefore reflects a balance between diversity of Interest and locale and compactness of size, and convenience of assembly. Doubtless we could profit by special talents of other members, but the group should not be enlarged to the point where fraquent and easy assembly becomes difficult, or where frank and casual discussion is inhibited.

U. of Oregon:
Novick Biophysics

Cal. Inst. of Technology: Horowitz Biology

Horowitz Biology Stanford University:

Van Niel Microbiology Krasskopf Geochemistry Lederberg Genetics (recorder)

While many members doubtless came to the first meeting with some sense of amusement and fravolity, it is obvious that the group as a whole is anxious to devote itself to tackling the problems of biological exploration with earnest endeavour. Many of its members are recognized as leaders in their own scientific fields, and in their academic communities. They have many other responsibilities. Nevertheless, there was unanimous enthusiasm for the continuation of its studies, and for meetings at relatively frequent intervals for mutual education and discussion. For this purpose, and to bridge the gap between exploratory discussions and preliminary experiments on one side, and tangible proposals and instrumentation for payloads on the other, we will require a substantial measure and continuity of financial support. In this proposal, several grades of support are indicated for more and more comprehensive activities. While the last and largest items might be deferred pending the elaboration of more explicit proposals, we should have prompt verification of support for our current discussions.

Minutes of Westex's first meetings are appended. The first meeting (Westex-1) was mainly devoted to problems of policy in colestial contemination, in view of urgent needs for the CETEX-COSPAR meetings. In brief we concluded that a basic policy of rigorous decontamination of space probes was both essential and feasible, -- modern methods of sterilization having been overlooked in other discussions. At the second meeting, this policy was reaffirmed. We then heard from Sinton on Infra-red reflection spectra of Mars, which have furnished virtually conclusive evidence for 'vegetation'. We are digesting a number of ideas for improving the quality of this type of information from 'safe' (viz. distant approaches), and this will doubtless be the main topic for the near future. Finally we have in mind the careful preplanning of

experiments based on 'soft landings', especially on Mars, predicating these for about 1965. This will allow somewhat over two years for decisions on the most efficient types of experiments, leaving an equal length of time for the devalopment and testing of the corresponding instrumentation. This timetable while not oppressive still does not allow for an indefinite waste of time. If properly supported, perhaps this might be one program that can be pursued with reasonable diligence and care rather than frantic haste. There is of course the possibility that the schedule may be accelerated (or delayed) by unforeseen technical factors, or by the pressure of international compatition. Specifications for the vicinal probes are perhaps already under substantial pressure of time.

ORIGINAL PAGE IS OF POOR QUALITY Proposal: Westex (A)

Travel, Communications HANANANANANANA for continued meetings of the Westex Group. \$6,000 per year.

This is based on holding about 10 meetings per year at various locations. There is a substantial advantage in meeting at different places, not only for the convenience of its peripheral members, and to help assure their attendance, but also to make further contacts with other local scientists.

While the travel costs are reduced by our regional grouping, this rather facilitates our meeting more often and more effectively for a given appropriation. In addition, there are substantial telephone charges for related business—the more so to make the most effective use of frequent meetings. It is likely that not every member will be able to attend every meeting. On the other hand we would profit greatly by being able to invite occasional distant 'consultants'— e.g. Fred Sinton at Westex-2. Admittedly, the development of a field as novel (in the U.S.) as astrobiology requires some expense for just the education of the workers who may participate in it.

If permissible, some of these funds (actually an insubstantial sum) should be available for the purchase of reference materials for the use of Westex members. On the other hand, at this stage, the time of Westex members is made available without cost other than expenses.

ORIGINAL PAGE IS

Wastax (B)

Publication of background information; "Handbook of Planetary Biology"

From the first discussions with Dr. Rossi, it has been evident that a critical requirement for the participation of U.S. biologists in space research is the collection of background information in a convenient form. This would include resumés of the Westex and 'Eastex' meetings, the essentials of present and prospective vehicle capabilities, and the environment (in the vehicle) for experimentation, and a critical discussion from the biologist's standpoint of available information on the environment of interplanetary space and the various planets. Most of this Information can be found in the astronomical and other literature, but we know from our own experience how difficult it is for a biologist, who has not given much previous thought to extraterrestrial science, to acquire this background. For example many of our colleagues still believe that the capability for planetary probes is decades away (which, hoepfully is not true) so that it would be pointless for them to attend to this challenge. While some member of CETEX may be able to rob the time from his other duties to prepare such resumss, this really is a substantial job, and there is some problem in finding a sufficiently informed enthusiast to do the work. Fortunately Mr. Carl Sagan may be available for some months this summer, and perhaps again after he completes his dissertation in astronomy (planetary atmospheres) at the Yerkes Observatory. A xx proposed budget would be \$4,000 for a (part-time) salary ? to Mr. (later Dr.) Sagan as consultant to Westex, plus \$3,500 for incidental costs in secretarial work, duplication, travel, reference materials. Hr. Sagan might have several functions: a) in the preparation of the consolidated reports of Westex (and, with their approval) Eastex for, perhaps, journal publication; b) as an adviser to Westex, particularly in the review of existing literature, and c in the preparation of the more extensive handbook. This might have some 60-100 pp. The means of its dissemination is open to further discussion -- either informal distribution to some few hundred leading scientists, as a mimeographed bulletin, or publication by MASA or by a commercial publisher (which should not be difficult to arrange, if this is the best course).

Westex (C) Exploratory experiments. \$10,000 -- 50,000

The design of payload instruments will have to be backed up by a subtantial amount of laboratory work, since the am analytical methods are limited by restrictions on weight, closeness of approach, automation, and the communication bandwidths. For example, there is relatively little published information on infrared xaxax reflection spectra of various materials, as would be comparable to Sinton's measurements on Mars. Before any member of Westex commits his owntime and resources, to the point of preparing a detailed proposal of laboratory work involving large scale support, some exploratory observations should be made in one or another laboratory, or perhaps most convaniently on a subcontract basis with some institution such as Stanford Research Institute. A for example, the evaluation of Sinton's work, and its use as the basis for vicinal probes, would be greatly facilitated by the measurement of diffusa reflection spectra from model spheres coated with various substances (e.g., cellulose; pastes of photosynthetic bacteria). Unquestionably many similar questions will arise (and have arisen). It would be most expeditious if funds were available to help support exploratory trials on points which arise in our discussions. While, in terms of this proposal, these would be administered by one responsible grantee (Stanford University) it is understood that these would be available for expenditures at other institutions as will give the most expeditious results in these preliminary stages. Further devalopment will be on the initiative of a scientist who undertakes the responsibility for pursuing a particular program, and will prepare his own budget request for this.

The scope of these explorations will probably be influenced by the funds that NASA is prepared to offer for them. I can visualize effective use of at least \$10,000 per year for a rather limited scale, or perhaps \$50,000 if we can have some leaway in purchase of equipment and in preliminary instrumentation towards payload designs. Any more extensive expedditures should certainly be made on the basis of explicit projects, following these explorations. These are, of course, uncommonly expensive as they require the development of new equipment modifications; in any case, even commercially available equipment in the particular field of molecular spectroscopy is far from inexpensive.

D. Possible Stanford projects.

This is a preliminary statement concerning the possible continuation of Sazan's present work after he completes his dissertation at Yerkes. He has been studying the spectra of the major planets and is interested in the identification of some lines with more complex molecules, e.g., amino acids, as must be expected to be formed photochemically on the basis of Miller's experiments. He is interested in further model experiments on the extent of organic accumulation, especially in gravitational fieldswhich has an important bearing on the possibility of organic sediments, e.g., under the Jovian oceans. This is precisely the same work as is needed to support experimental designs in ultimate probes to these planets, and it fits very closely with the Martian models, in which he is no less interested. I would propose to use the opportunity of Sagan's work as a consultant to Westex (proposal B) to lay the groundwork for a more detailed proposal. This would doubtless appear as an application over his own signature as responsible investigator, though I would support this in every way possible.

This statement is made to illustrate one way in which interim support for our group can help to build up momentum for research in planetary biology.

As concerns my cwn participation, apart from recording these conferences and exciting the interest of my (sometimes still diffident) colleagues in biology, I would feel most at home in any personal laboratory work in contact, rather than vicinal, experiments. If we develop an interestional policy of space exploration that assures an uncontaminated field of exploration, I should be interested in developing techniques of cultivation and assay for use with soft landings. In view of the indicated timetable, I would not need special financial support for another two or three years, especially if some exploratory resources are available from proposal C.

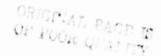
J. Lederberg

Dacamber 10, 1959

Dr. Robert Jastrow Chief, Theoretical Division National Aeronautics and Space Administration 8719 Colesville Road Silver Spring, Maryland

Dear Bob:

: As I indicated in my memorandum of November 25th, I am willing and interested to see what I can do mysalf by way of devaloping some of the experimentation directed towards the detection of planetary life. In view of the problems that NASA has faced in organizing its exobiological program, I have asked the Rockefeller Foundation for some preliminary money and they have agreed to let us have \$10,000. This will not carry us very far but it will at least let us buy some of the equipment we need to break into the problem. I had thought that we could very well use not less than \$25,000 during 1960 for the most primitive developmental work. I had asked Rockefeller for this full amount but they evidently do not feel ready to carry the full burden. Certainly by next summer and possibly much sooner, the availability of funds will be a serious limiting factor for the continuation of our work in this field. I would very much appreciate your advice on the prospects of obtaining this support from NASA as of the start of fiscal 1961. I believe that my memorandum should give enough information to support this quary. Until NASA has both the funds and the review machinery to make grants in this area, it would be pointless for me to prepare a more elaborate prospectus. I rely on your advice as to when it will be worth spending the time Roughly speaking, and if and effort to write up such a project. all goes reasonably well, I would guess that we would ask for about \$40,000 a year for fiscal 1961, 1962, and 1963. The exact amounts regulred, especially in the later phases of the program, may depend on the extent to which we can interpolate our instrument into other projects. For example, it will doubtless be necessary to use a video-tape recorder unit for several purposes in planetary instrumentation. The budget I have Indicated probably could not even encompass the purchase of such a unit, let alone the necessary development. The Ampex Corporation tells me that their commercial TV recording system now costs \$52,000 for the basic unit and is, of course, quite prohibitively massive for our intended application. For the most praliminary stages, however, I am confident that we could get their cooperation in using their equipment for tentative trials. Meanwhile, there will have to be



an independent development of such apparatus for use in space probes. Among the information we would have to develop would be the minimum requirements of such a recording (and transmitting) system to do an effective job of biological detection. This aspect of the work has to be done in a biological laboratory such as ours.

Yours sincaraly,

Joshua Lederberg Professor of Genetics

Space Science Board, National Academy of Sciences

TEN YEAR PROGRAM IN SPACE SCIENCE

(Submitted by J. Lederberg 10/10/59)

Suggestions for planetary biology.

The detection and characterization of life beyond the earth is one of the principal aims of spaceflight research. To a greater extent than in the physical sciences, it is difficult to plan in detail beyond the initial steps, the information from which would be crucial for subsequent plans. The conclusions of planetary chemistry are especially important as a foundation for biological work. On the other hand pioneering studies must be conducted so as not to prejudice later efforts. Explicit recognition of aims in planetary biology is therefore needed, even in advance of detailed experimental planning.

The following outline is predicated on the following possibilities for vehicular and communications development:

The satellite telescope (in orbit around earth)

Planetary probes:

Mars, Venus, Jupiter, Moon vicinal approaches

Moon; Mars-Venus? orbiters Moon, Mars, Venus controlled landings

landing and return (unmanned) Moon

Program (in rough sequence only)

- 1. Telescopic observations of Mars and Venus for organic substances in atmospheres or surface material.
- 2. Ditto, by vicinal approach (this competes with <1> if closer approaches, and consequent advantages in energy collection and angular orientation (i.e. size of collecting lens and its stabilization), compensate for costs of propulsion and guidance.
- 3. Orbiters: high resolution optical (including IR) surveys; vidicon photography with resolution of order of .1 to 1 meter might give evidence of vegetation. Applied to Moon, this would be important in choosing advantageous sites for chemical analysis, e.g. plutonic emissions.
- 4. Controlled landings. For the first time, these will permit explicit biological searches. In general, chemical analysis would precede any comprehensive biological survey.

Moon: Chemical and physical studies should take full precedence. Very large scale surveys would be necessary to detect the remotest possibility of dormant life at any point near the surface. Singularities in surface composition would be possible leads for biological followup in ultimate experimentation.

Mars and Venus: Larger organisms might be detected by their form or movement by vidicon surveys. Fore likely, a microscope input to the vidicon could detect microorganisms in the 'soil' and air-borne dust, either as such, or after nutrition with water and other substances. The same instrument could be used for simple cytochemical procedures to identify DNA and other Important

components of terrestrial life. These biological surveys should be concurrent with chemical analysis, which should include tests for organic molecules. The spectrophotometer and mass spectrometer would be useful as terminal sensors for systems of biochemical analysis. Automatic culturing devices could increase the availability of particular kinds of microorganisms, e.g., phototrophs, for cytochemical analysis.

5. Return samples (Moon). For any scientific purposes, these should be hermetically sealed to avoid exposure to Earth's atmosphere upon re-entry. Fortunately, this precaution will also protect the samples for biological analysis. One can suggest some far-fetched possibilities -- e.g. search for spores or traces of DNA -- but probably it would be more reasonable to complete preliminary chemical studies before programming the biological work.

Even when it becomes technically possible, samples should <u>not</u> be returned from other planets until the consequences of possible biological contamination of the Earth have been exhaustively studied with the help of remote, telemetric instrumentation.

It is doubtful whether the question of return of manned flights to the planets will arise during the 10 year interval.

The interplanetary medium. Samples of interplanetary particles (collected from fine space as well as on the lunar surfaces) will certainly be sought for chemical analysis. These should be collected in such a way as to conserve carbonaceous molecules that would be of biochemical interest. This qualification will make it necessary to develop new collecting devices avoiding he use of grease, paper, millipore filter membranes, and similar articles in current practice.

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EXOBIOLOGY, EXPERIMENTAL APPROACHES TO LIFE BEYOND THE EARTH

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Abstract: The detection of life beyond the earth is one of the most exciting challenges of space science. The problems of exobiology have important implications for the development of theoretical biology and the understanding of mechanism of the evolution of life, as well as for general philosophical conceptions of man's place in the universe.

The critical techniques of actual exobiological experimentation are believed to be those of microbiology, since micro-organisms for several reasons have a most important place in our consideration of program policy and in the solution of basic biochemical problems. Among the most important of these is the role of nucleic acids and proteins in the functioning of any organisms that may have evolved on other sites.

The rapid growth of micro-organisms, and the variety of their adaptations to different environments, dictate the most rigorous care to prevent the unwanted transfer of contaminants from one planet to another.

PESIOME: Поиски жизни вне земли являются одной из самых захватывающих задач наухи о космосе. Вопросы по внеземной биологии имеют большое влияние на развитие теоретической биологии и понимание механизма эволюции жизни, также как и для общей философической концепции места занимаемого в мире человеком.

Считается, что важнейшей техникой современных внеземных биологических опытов является микробиология, ввиду того, что микроорганизмы занимают по многим причинам главное место, принимая во внимание программу и решение основных биохимических задач; одной из самых важных из них является роль нуклеиновых кислот и протеинов в функционировании каждого организма, могущего развиться в других областях пространства.

Быстрое развитие микроорганизмов и разновидная приспособляемость к различным условиям, указывают на необходимость принятия строгих мер к предотвращению нежелательного перемещения заразных частиц с одной планеты на другую.

Résumé: La détection de la vie hors de la terre est l'une des questions les plus intéressantes de la science spatiale. Ce problème est d'une grande portée pour les fondations de la biologie théorique, et aussi pour le concept philosophique de la place de l'homme dans l'univers.

Les techniques principales de l'exobiologie doivent venir de la microbiologie terrestre puisque les microorganismes occupent une place importante dans la

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programme d'exploration de l'espace, et pour la résolution des questions fondamentales de la chimie biologique, par exemple, le rôle des acides nucléiques et des protéines dans l'économie vitale des organismes des autres mondes.

La croissance rapide des microbes, la diversité de leur possibilité d'adaptation aux differents milieux, exigent les plus rigoureuses précautions pour éviter le conta-

mination entre les corps celestes.

It is a privilege to discuss some basic problems in biology with an audience whose special concern is for the recent striking advances in the physics of the earth in the solar system. However, many of us are looking forward to the close investigation of the planets and few inquisitive minds can fail to be intrigued by what these studies will tell of the cosmic distribution of life. To conform to the best of our contemporary science, much thoughtful insight, meticulous planning, and laboratory testing must still be invested in the experimental approaches to this problem. This may require international cooperation and also, perhaps more difficult, mutual understanding among scientific disciplines as isolated as biochemical genetics and planetary astronomy.

Many discussions of space exploration have assumed that exobiological studies might await the full development of the technology for manned space flight and for the return of planetary samples to the terrestrial laboratory. To be sure, these might be preceded by some casual experiments on some instrumented landings. One advantage of such a program is that time would allow exobiological experiments to be planned with composure and deliberation. Undoubtedly, this planning would be more rigorous insofar as it was based on improved knowledge, from closer approaches, of the chemistry and physics of planetary habitats. Unfortunately, this orderly and otherwise desirable program takes insufficient account of the capacity of living organisms to grow and spread throughout a new environment. This unique capacity of life which engages our deepest interest also generates our gravest concerns in the scientific management of missions beyond the earth. On account of these, as well as of the immense costs of interplanetary communication, we are obliged to weigh the most productive experiments that we can do by remote instrumentation in early flights whether or not manned space flight eventually plays a role in scientific exploration.

Motivations for exobiological research

The demons which lurk beyond the Pillars of Hercules have colored the folklore and literature of ages past and present, not always to the benefit of fruitful exploration and dispassionate scientific analysis. Apart from such

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adventuresome amusements and the amateur delights of a cosmically enlarged natural history, how does exobiology relate to contemporary science and culture? The exploration of space may seem to have very little to do with fundamental questions in biology or medicine, with the role of genes and embryological development, protein synthesis, the biology of viruses or the evolution of species. To answer this question we may consider one aspect of the history of the physical sciences. Twenty-five centuries of scientific astronomy have widened the horizons of the physical world and the casual place of the planet Earth in the expanding universe is a central theme in our modern scientific culture. The dynamics of celestial bodies, as can be observed from the earth, is the richest inspiration for the generalization of our concepts of mass and energy throughout the universe. The spectra of the stars likewise testify to the universality of our concepts in chemistry. But biology has lacked tools of such extension, and life until now has meant only terrestrial life. This disparity in the domains of the physical versus the biological sciences attenuates most of our efforts at the construction of a theoretical biology as a cognate of theoretical physics or chemistry. For the most part, biological science has been the rationalization of particular facts and we have had all too limited a basis for the construction and testing of meaningful axioms to support a theory of life. At present, perhaps the only potentially universal principle in biology is the Darwinian concept of evolution through the natural selection of random hereditary fluctuations.

Some chemical attributes of terrestrial life might support a claim to be basic principles: for example polyphosphates (adenylpyrophosphate) occur in all organisms as coupling agents for the storage and transfer of metabolic energy. But at least in principle, we can imagine that organisms may have found alternative solutions to the same problem. Only the perspective of comparative biology on a cosmic scale could tell whether this device is an indispensable element of all life or a particular attribute of its local occurrence on this planet.

An important aim of theoretical biology is an abstract definition of life. Our only consensus so far is that such a definition must be arbitrary. If life has gradually evolved from inanimate matter, the demarcation of chemical from biological evolution is one of useful judgment. For a working principle, we might again rely upon the evolutionary concept: a living system has those properties (of self-replication and metabolism) from which we may with more or less confidence deduce an evolutionary scheme that would encompass self-evidently living organisms. But I do not propose this as a rote formula for the assessment of other celestial bodies and certainly not before we have some empirical knowledge of the diversities of chemical evolution.

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From this standpoint the overriding objective of exobiological research is to compare the over-all patterns of chemical evolution of the planets, stressing those features which are globally characteristic of each of them.

We are all thinking of the question: "Is there life on Mars?" To answer it may require a careful reassessment of our meaning of "life" and matching this with the accumulation of hard-won evidence on chemical composition of that planet. On the other hand, we might be confronted with an object obviously analogous to an earthly plant, animal or microbe. But even this abript answer would be trivial in deference to a biochemical analysis of the organism and its habitat for comparison with the fundamentals of terrestrial life.

In our first approaches to the nearby planets we will wish to design experiments which have some tangible foundation in the present accumulation of biochemical knowledge. The aqueous environment, and its corollary of moderate temperatures in which large carbonaceous molecules are reasonably stable, are implicit in terrestrial biochemistry. This is not to reject the abstract possibility of nonaqueous life, or noncarbonaceous molecules that might characterize temperatures of <200 or >500° K. However, we can defer our concern for such exotic biological systems until we have gotten full value from our searches for the more familiar, and have learned enough of the exotic chemistry to judge how to proceed.

Within the bounds of its aqueous environment, what are the most nearly universal features of terrestrial life? In fact, our plants, animals and bacteria share a remarkable list of biochemical components and a biochemist cannot easily distinguish extracts of yeast cells and beef muscle. Among these components, the nucleic acids warrant first attention. Although they constitute the hereditary material, so that all the variety of terrestrial life can be referred to subtle differences in the nucleic acids, the same basic structure is found in the nuclei of all cells. This is a long, linear polymer fabricated from a sugar-phosphate repeating unit:

The meaningful variety of nucleic acids depends on the specific order of the side-group attached to each sugar or this monotonous backbone, a linear message written in a language of four letters, T, C, A, G. The bacteria, which are the simplest free-living organisms, contain nucleotide sequences about 5 000 000 units long; man has about 5 000 000 000 — this content being one of our best, objective measures of biological complexity. On the other hand, the simplest viruses, which can multiply only inside living cells and come close to being single genes, have about 2500 units per particle. Playing a central role in the unification of terrestrial biology, nucleic acids underly both heredity and, (through their control of protein synthesis) development. Are they the only linear polymers which can subsume these functions, or will many other fundamental types have evolved, to be found on other celestial bodies?

Equally general among the constituents of living cells are the proteins, which are also polymers, but of a more diverse set of constituents, some 20 amino acids. The fundamental backbone of a protein is a poly-amino acid chain:

where R may be any of twenty different groups, distinguishing a like number of amino acids found in natural proteins. Proteins assume a wide variety of three-dimensional shapes, through coiling and cross-linking of the polymer chains. They are in this way suited to perform such diverse functions as those of enzymes, structural elements, and antibodies. Not only do we find just the same 20 amino acids among the proteins of all terrestrial organisms, but these are all the levo-isomers, although dextro-amino acids are found to have other metabolic functions. Next only to the incidence of nucleic acids, we would ask whether exobiota make analogous use of proteins, comprising the same amino acids, in hopes of understanding what seem to be random choices in the sculpture of our own living form.

Common to terrestrial life are also a number of smaller molecules which are involved in the working metabolism of the cells; for example most of the B vitamins have a perfectly general distribution. They are vitamins for us only because we have learned, in our evolutionary history, to rely on their production by green plants, rather than to synthesize them within our own cells. But once formed, these vitamins, and similar categories of substances such as porphyrins, play entirely analogous roles in the metabolism of all cells.

A few substances, such as the steroid hormones, do play special roles in the metabolism of higher organisms, and testify to some progress in biochemical

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evolution. In fact, most objective evidence points to a loss of specific functions — microorganisms are certainly more versatile and less dependent than man is on a specific nutrient milieu. The main burden of evolution from the prototypes of one to the other has not been to develop new biochemical unit processes but to coordinate them in time and space.

While we propose to give first priority to these most general questions, they by no means exhaust our interest in the pecularities of extra-terrestrial organisms, any more than they would for a newly discovered phylum of the earth's own repertoire. Nor should we even preclude the possibility of finding new organisms that might be economically useful to man, just as they were among the most fruitful yields of geographic exploration. However, the enlargement of our understanding, rather than of our zoos and botanical gardens is surely our first objective.

Theories of the origin of life

At this point, a consideration of contemporary theory on the origin of life is justified for two reasons: (1) exobiological research gives us a unique, fresh approach to this problem, and (2) we can find some basis to conclude that life need not be so improbable an evolutionary development as had once been speculated.

The interval between Pasteur's work on spontaneous generation and the recent past has been especially difficult for the mechanistic interpretation of the origin of life. Before Pasteur's time, many investigators could believe that simple microorganisms arose spontaneously in nutrient media. His demonstration that such media remained sterile if properly sterilized and protected seemed to disqualify any possibility of "spontaneous generation". His conclusion was of course overdrawn, since life must have evolved at least once, and the event could still occur though very much less frequently than had been supposed before. Meanwhile, the problem was compounded by the growth of biochemical knowledge. We now realize that bacteria, as small as they are, are still extremely complex, well-ordered and representative organisms. The first organisms must have been far simpler than present-day free-living bacteria.

With the growth of genetics since 1900, and the recognition of the self-replicating gene as the elementary basis of life, the question could focus on the origin of the first genetic molecule: given the power of self-replication, and incidents of stochastic variation, Darwin's principle could account for the eventual emergence of any degree of biological complexity.

An immense amount of fruitful genetic work has been done in a period

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when "genetic molecule" was an abstraction and "self replication" an axiomatic principle whose chemical basis seemed beyond the possibility of human understanding. Now we recognize that the nucleic acids are the material basis of heredity and can begin to construct mechanistic models of their replication. The first principle, as already stated, is that the gene is a string of nucleotides each position in the string being marked by one of the four nucleotide units A, T, C, and G. The polymerization of such strings by the union of the monomeric units presents no fundamental problems, but self-replication would necessitate the assembly of the units in a specific order, the one dictated by the order of the nucleotides in the parent molecule. The key to the solution of this problem was the realization by Watson and Crick that the complete nucleic acid molecule is a rigid, duplex structure in which two strings are united. In that rigid structure, as can be shown by suitable molecular models, adenine occupies a space which is just complementary to that of thymine, and cytosine is likewise complementary to guanine. A string can therefore replicate, i.e. direct the assembly of another daughter string, in the following way. The nutrient mix of the cell contains all four nucleotide units. However, at any position of the parent nucleic acid molecule only one of these four can make a suitable fit and will therefore be accepted. After being accepted, the daughter units are firmly bound together by new chemical linkages giving a well-defined daughter string. Kornberg has reconstructed most of these events in some detail, by means of extracts from bacteria, to the very verge of proven duplication of genes in a chemically defined system in the test tube.

However, the media in which such syntheses can occur, in the cell or even in the test tube, are extremely complex. Given that the simplest organisms would be the most dependent on their environments for raw materials, where did these precursors come from before living organisms had evolved the enzymes to manufacture them?

Thanks to the insight of Haldane, Oparin, Horowitz and others, we now realize that this paradox is a false one, though it dates to the confusion between "carbon chemistry" and "organic chemistry" which still exists in English terminology. In fact, in 1828, Wöhler had already shown that an organic compound, urea, could be formed experimentally from an inorganic salt, ammonium cyanate. A hundred years later, a number of routes for synthesis of geochemically significant amounts of complex organic materials were pointed out, for example the hydrolysis of metallic carbides, and subsequent reactions of olefins with water and ammonia. More recently, Miller and Urey demonstrated the actual production of amino acids by the action of electric discharges on gas mixtures containing the hydrides NH₃, OH₂, and

CH₄. This demonstration converges with other arguments that the primitive atmosphere of the earth had just such a reduced composition, becoming oxidised secondarily (and in part through photosynthetic separation of C from O₂).

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An alternative origin of carbonaceous molecules is even more pervasive. Perhaps we associate carbon with life, and rocks and metals with physical phenomena — beyond doubt we tend to connote the latter with the predominant substance of the universe. In fact, as a glance at tables of cosmic abundance will show, the lighter elements by far are the most prevalent and after the dispersed H and He these are C, O and N. The primitive condensation of free atoms to form the interstellar smoke, and eventually the stars themselves, must entail the molecular aggregation of H+C+O+N; i.e., a large fraction of the condensed mass of the universe must consist, or once have consisted, of organic macromolecules of great complexity. The chief problem for their synthesis is in fact not a source of chemical energy but how to dissipate the excess energy of reactions of free atoms and radicals.

This aspect of astrophysics may have place for a remote biological analogy: once a few molecules have formed, the energy of subsequent impacts can be dissipated among the vibrational degrees of freedom. That is, such molecules can function as nuclei of condensation. Those molecules will be favored, as seeds for further condensation which (1) most readily dissipate the energy of successive impacts and (2) can undergo molecular fission to increase the number of nuclei. The actual molecular chemistry of the interstellar (or prestellar) smoke is thus subject to a kind of natural selection and cannot be a purely random sampling of available atoms.

Whether the earth has retained remnants of this chemistry is hard to say. There is at least some evidence of it in the spectra of comets, and fragments from these continue to form part of the meteoroidal infall. These particles, unless associated with larger meteorites, would be unrecognizable after traversing the earth's atmosphere — they are among the possible treasures to find buried in protected crevices on the moon.

Light traversing the interstellar smoke has been found to be polarized. If primitive aggregation plays some role in furnishing precursors for biological evolution, this polarization furnishes at least one bias for a decision between levo- and dextro-isomers.

At any rate, possible sources for probiotic nutrition no longer pose a problem. Before the appearance of voracious organisms, organic compounds would accumulate until they reached equilibrium with thermal and radiative decomposition, from which the oceans would furnish ample cover. Locally, the concentration of the soup would be augmented by selective evaporation,

and by adsorption onto other minerals. The main gap in the theory, not yet bridged by any experiment, is the actual formation of a replicating polymer in such a morass. We are beginning to visualize the essential conditions for chemical replication and its ultimate realization is foreshadowed both by biochemical studies of nucleic acids and by industrial syntheses of stereospecific polymers.

There is some controversy whether nucleic acids were the first genes, partly because they are so complex, partly because their perfection hints at an interval of chemical evolution rather than one master stroke. The advantage of the nucleic hypothesis is that no other self-replicating polymers have so far been found. But as an alternative speculation a simplified protein might replicate by the complementary attachment of acidic vs. basic units, perhaps the crudest possible method of assembly. The nucleic acids would be perfections on this theme for replication. The existent proteins do not replicate; with their variety of amino acids, they would have evolved as better adaptations for assuming specific shapes. A comparative view of independent evolutionary systems may at least serve to check such speculations.

Although many steps in the generation of living molecules remain to be recreated, we can state this as a relevant problem for exobiological study, with considerable optimism for the prevalence of life elsewhere. A sterile planet, too, would be of extraordinary interest to biology for the insight it should give on the actual progress of probiotic chemical evolution.

Natural and artificial panspermia

The foregoing discussion tacitly assumed that the evolution of planetary life was a local phenomenon, independent of its incidence elsewhere. But, at a time when de novo generation seemed less plausible than it does now, Arrhenius defended another hypothesis: panspermia, the migration of spores through space from one planet to another. The credibility of the panspermia hypothesis has been eroded mainly for two reasons: (1) the lack of a plausible natural mechanism for impelling a spore-bearing particle out of the gravitational field of a planet as large as the Earth, or any planet large enough to sustain a significant atmosphere and (2) the vulnerability of such a particle to destruction by solar radiation. In any case, panspermia could be disparaged for evading the fundamental problem, by transposing it to an unknown, perhaps scientifically unknowable site. These difficulties have impeached the standing of panspermia as an experimentally useful hypothesis, but not its immense significance for cosmic biology. In its defense, it might be indicated that the hazards of exposure to space may be exaggerated, taking account

of the dormancy of microorganisms in high vacuum and low temperatures, and their relatively low cross-section for ionizing radiations. The chief hazard to microorganisms might come from solar ultraviolet and the proton wind, but a thin layer of overlying material would shield a spore from these. For the impulsion of particles we might possible appeal to impacts with other heliocentric bodies, be they grazing meteorites or planetoids in cataclysmic encounters - suggestions not more remote than those invoked for other astronomical phenomena. Nor can we be sure that all the electrokinetic mechanisms which Arrhenius may have had in mind can be excluded from applying to any single particle. In testing for panspermia, we would be concerned first of all for evidence of interplanetary transport of any material. The moon suggests itself as a nearby trap for particles of terrestrial origin among which living spores or biochemical fragments of them might be the most characteristic markers. At one spore per kilogram of sample, a weight ratio of 10-15, the sensitivity of easy biological detection partly compensates for the vulnerability of spores to physical hazards.

The development of rocket-impelled spacecraft has, of course, furnished a mechanism for artificial panspermia. Several authors have recently revived Haldane's passing suggestion that life might even have been disseminated by intelligent beings from other stellar systems. That another century of productive science and technology could give the human species this capability would be hard to dispute. The hypothesis is connected with the age or agelessness of the universe - until we have a basis for decision on this point, and can make independent tests for intelligent life elsewhere it must join natural panspermia in the limbo of irrefutable, untestable scientific hypotheses. The technique for attempted radio communication with nearby stars has been

detailed recently by Cocconi and Morrison.

These new tools for the exploration of the universe have caught many of us unawares, and few can pretend to have recaptured their equilibrium in dealing with these concepts. Irrefutable notions have little scientific value unless they lead to attempts at verification. A priori arguments for the presence or absence of intelligent life on the planets or in nearby stellar systems are equally unconvincing. The skepticism of most scientists is justified not by conviction but by the consistency of negative evidence in the limited scientific data that have so far been collected.

Planetary targets

The suitability for life of the accessible bodies of the solar system has already received ample attention. Mars is, of course, the likeliest target, most nearly resembling the habitat of the earth. The indicated scarcity of free moisture and oxygen would severcly limit the occupation of Mars by man or most terrestrial animals. However, there seems little doubt that many simpler, earthly organisms could thrive there. Indeed, many students have concluded that Mars does have a biota of its own. The most pertinent evidence is perhaps the infra-red reflection spectrum recorded by Sinton which indicates an accumulation of hydro-carbonaceous materials in the dark areas. This is complemented by Dollfus' report (cf. this Symposium) on the seasonal changes of granularity of these areas. The main reservation that must be registered is that these might be meteorological phenomena involving masses of material which may be carbonaceous but not necessarily living. Most such material on the earth's surface is associated with life. However, this may be connected with the greedy utilization of such compounds by organisms rather than their production by vital synthesis. However, the most plausible explanation of the astronomical data is that Mars is a living planet.*

The habitability of Venus is connected with its temperature, a highly controversial subject. Perhaps the most useful first contribution to the exobiology of this planet would be a definitive measurement of its temperature profile. Even should the surface be unbearably hot, this need not preclude a

more temperate zone at another layer.

The exposure of the moon's surface to solar radiation and its absence of a significant atmosphere have discounted the possibility of a lunar biology. However, the composition of the moon's deeper layers, from even a few meters beneath the surface, is very much an open question (cf. Urey, this Symposium) particularly in the light of Kozyrev's recent reports of gaseous emissions. Realistic plans for the biological study of the moon probably must await the results of chemical analyses. Apart from the remote possibility of indigenous life the moon is a gravitational trap for meteroidal material. We may eventually be able to screen large quantities of this virgin material for what Haldane called astroplankton — the empirical test of the panspermia hypothesis. While exposed deposits would be subject to solar degradation, shaded refuges must also exist. Mercury may be analogous to the moon, except insofar as its dark side may furnish an even more reliable, though much remoter, refuge of this kind.

It may be academic to discuss the exploration of the major planets in view of their distance and the difficulty of deceleration in the Jovian field. However, their wealth of light elements, subject to solar irradiation at temperatures

The term "vegetation" is often used—this should be discouraged if it implies
that the Martian biota will necessarily fall into the taxonomic divisions that we know
on earth.

and in gravitational fields very different from the earth's offers the most exciting prospects for novel biochemical systems.

Experimental approaches

Our treatment of this topic warrants the utmost humility from a realistic view of our limitations. Useful landings on planetary targets are fraught with difficulties and hazards; experiments to be conducted at some distance from their targets should not be overlooked in the excitement of planning for more adventurous missions. Balloon- and satellite- mounted telescopes can tell much about planetary chemistry and hence biology and probes to the vicinity of the targets can furnish additional information prior to actual landing.

It is instructive to ask ourselves how we might diagnose the existence of life on the earth from distant observations. If we may judge from the photographs so far obtained from high altitude rockets, we could hope to detect only large scale manifestations of organized culture — cities, roads, rockets. This reserve may not give due credit to the possibilities of high resolution photography and sensitive infra-red spectrometry, and reasonable implications from seasonal changes in the color and texture of terrain. However, we may conclude that distant approaches will be invaluable for preparatory chemical information, but probably will not be decisive for exobiological inferences. Even if we could more surely decide that the Martian cycle involved living organisms rather than inanimate chemical transformations we would still have little insight into the intimate biochemical details which are a major objective of exobiological research. On the other hand, like our own extensive deserts and deep waters, a planet could harbor an extensive biota that would defy detection from a distance.

Microorganisms, for many reasons, are the best prospects on which to concentrate marginal capabilities. They are more likely to flourish in a minimal environment than larger organisms. The microbes must also precede the macrobes in evolutionary sequence though we must not suppose that present day bacteria are necessarily very primitive. The earth is well endowed with both kinds of organisms; we can imagine another world with only microbes; but we cannot conceive of one lacking microbes if it bears any form of life at all. Likewise, taking the earth as a whole, we find that large organisms occupy only a small fraction of the surface. However, we can reasonably expect to find evidence of microscopic life in any drop of water, pinch of soil, or gust of wind. Given a limited sample for study, microbiological analysis will certainly give the most reliable diagnosis for the presence of life anywhere on the planet. By the same odds the greatest diversity of biochem-

an experimental program.

ical mechanisms will be represented among the microbiota of a small sample. Microbiological probes also offer distinct advantages for the collection and analysis of living material. Starting from a single particle, microbes can easily be cultivated within the confines of an experimental device. In this they remain accessible to physiological and chemical experiments that would be extremely cumbersome for larger organisms. (Compare, for example, the automatic instrumentation that would be needed to catch a mouse or an elephant and then to determine its nutritional requirements.) The techniques of cytochemistry as have already been developed for the chemical analysis of microscopic cells and organisms appear to be the most readily adaptable to automation and telemetric recording, an important advantage under the existing pressure of time, talent and cost. Important issues of policy cannot be decisively settled without factual information on the growth capacity of the microorganisms that might be exchanged among the planets. Accordingly, the methodological precedents in terrestrial science for exobiology are most evident in microbial biochemistry. The conceptual aims are equally close to those of biochemical genetics. Needless to say no other resource or objective of serious biological science can be neglected in the development of

Aside from the experimental designs, the pace of exobiological research may be regulated by advances in vehicular and guidance capabilities and data communication. In the expectation that these will remain in reasonable balance – for static or real time television communication with the planetary probe – the microscope may be the most efficient sensory instrument. The redundancy of a pictorial image is not altogether wasted: would we confide in a one-bit pulse from an efficient black box to answer our cosmic queries?

According to this experimental concept, the terminal microscope-vidicon chain must be supported by three types of development: 1) for collection and transport of speciment to the aperture of the microscope; 2) for cytochemical processing of the samples; 3) for protection of the device from environmental hazards, its apt location after landing, provision for illumination, focussing, and perhaps preliminary image selection. Detailed studies of these problems are only just under way, and the following suggestions are only tentative.

The easiest specimens to obtain may be atmospheric dust and samples of surface soil once the device has been landed. These are collected on a travelling ribbon of transparent tape which is thrown out and then rewound into the device. Larger samples, collected by a soil auger, could be subjected to a preliminary concentration of non-mineral components by flotation in a dense liquid. The use of such a tape simplifies the problem of treating the samples

C-5

with a succession of reagents, for example specific enzymes and fluorescent stains which allow for the detection of nucleic acids and proteins. Microscopy with ultraviolet light, particularly at 2600 and 2800 Å, owing to its selectivity for nucleic acids and proteins may be the most direct way to distinguish microorganisms from mineral particles. Generally speaking, the microscope can be adapted to many simple analytical procedures whose construction on a larger scale would present formidable problems for automatic technique.

The adaptation of the microscope system to a payload can be untertaken more realistically when laboratory prototypes have been built and tested. For example, we will have to decide between accurate pre-focussing of a microscope whose lenses and entry slit are mounted in a rigid structure and continuous control of focus by an optically controlled servo system (an innovation that would be far from useless in the biological laboratory). Fluorescent staining may facilitate automatic discrimination for conservation of radio power: the travelling ribbon can be stopped and the vidicon-transmitter activated just when a stained object is in view.

These preliminary experiments can indicate some of the general features of the planetary microbiota. The data they furnish will support more intensive studies of the growth characteristics, chemical composition and enzymatic capabilities of organisms cultivated on a larger scale. The interaction of these organisms with tissue cultures of animal cells can also be considered. From the results of these initial probes we can better deduce how to anticipate the long range consequences of the intercourse of planetary biota.

Conservation of natural resources

A corollary of interplanetary communication is the artificial dissemination of terrestrial life to new habitats. History shows how the exploitation of newly found resources has enriched the human experience; equally often we have seen great waste and needless misery follow from the thoughtless spread of disease and other ecological disturbances. The human species has a vital stake in the orderly, careful, and well-reasoned extension of the cosmic frontier; it will be a crucial measure of the maturity of our national consciences and their concern for posterity, how we react to the adventuresome and perplexing challenges of spaceflight.

The introduction of microbial life to a previously barren planet, or to one occupied by a less adapted form can result in the explosive growth of the implant with consequences of geochemical scope. With a generation time of 30 minutes, and easy dissemination by winds and currents, common bacteria could occupy a nutrient medium the size of the earth in a few days or weeks,

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being limited only by the exhaustion of available nutrients. It follows that we must *rigorously* exclude terrestrial contaminants from our spacecraft. This stricture must hold until we have acquired the factual information from which we can assuredly assess the detriments of free traffic, and whether these are small enough to warrant the relaxation of these controls.

At the present time, the most obvious values that would be threatened by contamination are scientific ones. The overgrowth of terrestrial bacteria on Mars would destroy an inestimable prize for the understanding of our own living nature. Even if an intemperate mission has not contaminated a planet, the threat of its having done so will confuse later studies, if earth-like organisms are then found. However, other values are in question. Quite apart from strictly scientific concerns, would we not deplore a heedless intrusion on other life systems? It would be rash to predict too narrowly the ways in which undisturbed planetary surfaces, their indigenous organisms, their molecular resources may ultimately serve human needs. If we have cause to prejudice these values, we surely would not wish to do so by inadvertence.

To do this effectively requires a nice appreciation for the ubiquity and durability of bacterial spores, which are well preserved in high vacua and at low temperatures, and are only rapidly destroyed when kept at temperatures over 160° C. It is probable that spacecraft can be disinfected by the conscientious application of gaseous disinfectants, especially ethylene oxide, but this will succeed only if the procedure is carried out meticulously and with controlled tests of its effectiveness. Sealed components, if found to be potential sources of contamination, can be disinfected by chemicals, prior to sealing, or subsequently by heat, or irradiation at very high doses. The technology of disinfection is an expert one, and personnel already experienced in it should be delegated supervisory control.

The assessment of this problem involves a concept of risk that has not always been perceptively realized. The hazards of spaceflight itself, or of hard impact, or the planetary environment *might* suffice to neutralize any contaminants: but can we afford to rely on any uncertain suppositions when the stakes are so high, and when we have practical means at hand for conservative protection? We must be especially sensitive to the extreme variations in the environments of spacecraft or of planetary surfaces which might furnish refuges for microbial survival no matter how hostile the *average* conditions.

The indications by agencies both in the U.S. and the U.S.S.R. that adequate precautions will be exercised on all relevant missions are an important step in the realization of constructive exobiology.

Scientists everywhere will call for the application of these measures with

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the same care and enthusiasm as the more positive, exciting and patently rewarding aspects of space research. Scientific microbiology in the laboratory is absolutely dependent on the rigorous application of the special technique of pure culture with aseptic control. If we do not exercise the same rigor in space science we might as well save ourselves the trouble of thinking about and planning for exobiological research.

While early traffic to the planets will be one-way, we must anticipate the capability of roundtrip and even of manned spaceflight. Undoubtedly, planetary samples can be analysed for any scientific purpose more conveniently and more exactly in the terrestrial laboratory than by remote devices. For each step of analysis, special devices can be used (or if need be newly designed and constructed) and a constant give-and-take between human judgment and instrumental datum is possible. However, the return of such samples to the earth exposes as to a hazard of contamination by foreign organisms. Since we are not yet quite certain of the real existence of planetary (i.e. Martian) organisms, and know nothing of their properties, it is extremely difficult to assess the risk of the event. The most dramatic hazard would be the introduction of a new disease, imperilling human health. What we know of the biology of infection makes this an extremely doubtful possibility - most disease-producing organisms must evolve very elaborate adaptations to enable them to resist the active defences of the human body, to attack our cells, and to pass from one person to another. That a microorganism should have evolved such a capacity in the absence of experience with human hosts or similar organisms seems quite unlikely. However, a converse argument can also be put, that we have evolved our specific defences against terrestrial bacteria, and we might be less capable of coping with organisms that lacked the proteins and carbohydrates by which they could be recognized as foreign. Furthermore, a few diseases are already known (e.g. psittacosis, botulism, aspergillosis) whose involvement of man seems to be a biological accident. These arguments can only be resolved by more explicit data. Nonetheless, if they are harmful at all, exobiota are more likely to be weeds than parasites, to act on our agriculture and the general comfort of our environment, and to be pervasive nuisances than acute aggressors. However, even the remotest risk of pandemic disease, and the greater likelihood of serious economic nuisance, must dictate a stringent embargo on the premature return of planetary samples, or craft that might inadvertently carry them. Again, our preliminary experiments must give us the foundation of knowledge to cope with exo-organisms, even select those which may be of economic benefit. A parallel development of technique for disinfection may mitigate some of these problems - at present the prospects for treating a

returning vehicle to neutralize any possible hazard are at best marginal by comparison with the immensity of the risks.

Of the possible payloads for interplanetary travel, living man of course excites the widest popular interest. In due course, he may be supported by a sufficient payload to accomplish useful tasks in exploration beyond the capacities of instrumentation. However, he is a teeming reservoir of microbial contamination, the most difficult to neutralize, and an especially apt vehicle for infectious organisms. In view of these difficulties, and insofar as manned spaceflight is predicated on the return of the crew, a sound basis of scientific knowledge from instrumented experiments is a sine qua non for the planning of such missions.

Timely effort now to devise and build instrumented experiments is essential to keep pace with the technical capacities of space vehicles.

Concluding remarks and acknowledgment

Many of the ideas presented in this statement are not new. In the scientific literature, they have been treated only occasionally, for example in a remarkable article by J. B. S. Haldane (1954). They are also anticipated in the classic works of science fiction, e.g., H. G. Wells' "War of the Worlds", and by a flood of derivative fantasies of less certain quality either as science or as fiction. This kind of attention has not necessarily helped the realistic evaluation of the biological aspects of space travel which may still be dismissed as over-imaginative by some of our colleagues. However, exobiology is no more fantastic than is the realization of space travel itself and we have a grave responsibility to explore its implications for science and for human welfare with our best scientific insights and knowledge.

The principles embodied in this paper reflect the judgment of one among several of the scientific groups advisory to the Space Science Board of the U.S. National Academy of Sciences. However, they do not necessarily represent any official policy or the committed views of each consultant. The continued interest and advice of M. C. Ivi., R. Davies, N. Horowitz, S. E. Luria, A. G. Marr, D. Mazia, A. Novick, C. Sagen, G. Stent, H. C. Urey, C. B. van Niel, and H. Weaver, among many others, have been indispensable.

Literature

To document this article in detail with references to original sources would require a bibliography of inordinate length. Many of the issues are reviewed in the following works.

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NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

BELTSVILLE SPACE CENTER 4555 OVERLOOK AVENUE, S.W. WASHINGTON 25, D. C.

SPACE SCIENCES DIVISION THEORETICAL DIVISION VANGUARD DIVISION January 28, 1960

TELEPHONE: JOHNSON 2-6610 TWX: WA 134

Reply to: "Theoretical Division 8719 Colesville Road Silver Spring, Md.

Prof. Joshua Lederberg Department of Genetics Stanford University Palo Alto, California

Dear Joshaa:

I am not sure who has the ball as the result of our conversation in Nice, but if I am the one, then I should like to say that I would strongly support a proposal from you for the support of developmental work on planetary biology experiments, along the lines of your letter of December 10 and our subsequent conversation. The proposal should include a general description of your objectives and the motivation for the proposed research and instrumentation development, and whatever specific lines of attack which you may have in mind at this early stage. I would suggest that the communication be addressed to Homer Newell, with a copy to me.

With best wishes,

Sincerely,

Robert Jastrow

RJ:1s

Prof. J. Legerberg
Department of Genetics
School of Medicine
Stanford University
Palo Alto, California

February 2, 1960

Dr. Homer Newell
Deputy Director for Space Sciences
National Aeronautics and Space Administration
Washington 25, D.C.

Dear Dr. Newell:-

In previous conversation and correspondence with Dr. Jastrow and others of your colleagues, the urgancy of getting under way with a practical program in planetary blology has become quite evident. The problems of instrumentation and interpretation are so grave that we are much further behind in biology than the physical sciences were before the inception of a rocket, much less a planetary, program. Hy colleagues and I spent much of 1958 in delineating the problem, and 1959 in fixing on actual objectives and general approaches. We should not waste any more time than we must in designing, building and testing experimental devices. Dr. Jastrow and others have suggested that we should now submit actual proposals to elidt financial support for this work.

One of our serious problems is to obtain a realistic estimate of costs: this is almost a research project in itself, since one of our major aims is to define the kind of instrumentation that will be needed to detect planetary microorganisms. We also have to guess at our access to some quite expensive items, for casual use, on a courtesy basis, and which items we will have to have close at hand in the laboratory. For our formal proposal, i hope to be able to furnish a more explicit accounting. However, I would not wish to accept responsibility in this area for a program larger than about 25-35,000 dollars and propose to request such an amount for fiscal year 1961. Based on the findings we may make during this initial grant, we will make such further submissions as may then appear necessary for a successful conclusion of the project. This letter is an informal tender; I will be happy to have your suggestions and comments, and to discuss any ambiguitles, as the basis for submitting a formal application through university channels.

The main objectives of exobiological research are outlined in the attached paper; the enclosure also refers to the particular experiment of a planetary microscope for the detaction of extra-terrestrial microorganisms.

We do not propose to build such a device for spaceflight. The work in development and adaptation to do this should be done in close collaboration with an agency of the NASA such as the Jet Propulsion Laboratory. We do propose to acquire sufficient experience with a prototype vidicon-microscope system

that we can write precise specifications for a device that can answer our experimental questions. Necessarily, such specifications must not be too demanding in power or communications requirements, or in the weight and complexity of the operations. We propose, therefore, to set up a laboratory system that can simulate the quality of images we might hope to receive from a planetary transmission, and then to test this system on samples of terrestrial soil and dust. In addition, we will have to consider auxiliary devices to:

- 1.txam collect and transport samples to the microscope
- 2. concentrate microorganisms from sparsely populated soils
- 3. cultura microorganisms on segments of treated tapes
- 4. process the microorganisms in 'developing tanks' containing specific.
- 5. autofocus the microscope (presumably on monitor segments of the tape)
- 6. control tope transport and activate trabsmission when certain likely signals (e.g. a fluorescent-stained object) are in view

Barring a crash program, the development and testing of such devices should take two to three years. One year's work should enable us grasp the problem to the point where we know more definitely what we need, and can consider subcontracting some of the more obvious elements that have to be perfected. For this reason, we are now contemplating a one year grant.

To enable us to make an initial start without delay, we have received a grant of \$10,000 from the Rockefeller Foundation for calendar year 1960.

A tentative budget is attached.

Yours cordially,

Joshua Lederberg

Executive, Department of Genetics

CC: Dr. Jastrow, MASA
Dean Alway, Stanford Hedical School

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Tentativa Budget: Proposal to NASA

rom: Leland Stanford, Jr., University, Stanford, California (Department of Genetics, Nedical Center, Palo Alto, California)

CYTOCHEMICAL STUDIES OF PLANETARY MICROCREANISMS

Principal Investigator: Joshua Lederberg, Ph.D., Executive, Department of Genetics

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Professional and technical salaries (& CASI (excl. principal investigator) retint)	d 12,000
Equipment, purchase or construction	15,000
Supplies, expendable (laboratory, also Including office and reference)	3,000
Communications, secretarial and publication costs	1,500
Travel, for consultation on equipment and program planning	1,200
Subtotal, knd direct costs	32,700
indirect costs, at 25% of direct costs	8,175
(or other 5 as negotiated)	140,875

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION 1520 H STREET NORTHWEST WASHINGTON 25, D.C.

10 February 1960

TELEPHONE EXECUTIVE 3-3250 TWX: WA 755

IN REPLY REFER TO:

Dr. Joshua Lederberg Professor and Executive, Department of Genetics Stanford University Medical Center Palo Alto, California

Dear Dr. Lederberg:

It was indeed a pleasure to receive your letter of 2 February with an informal exploratory proposal to begin work on defining the kind of instrumentation that will be needed to detect planetary microorganisms. I am wholly in agreement that we should not waste any more time in getting under way in preparing a planetary biology program.

I am sending your letter to appropriate offices here at the NASA headquarters. We shall be in touch with you again in the near future.

Sincerely yours,

Momer E. Newell Deputy Director

Space Flight Programs

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION



NASA HEADQUARTERS
1520 H STREET NORTHWEST
WASHINGTON 25. D. C.
TELEPICANE EXSCUTIVE 3-3260 TWX: WA733

IN REPLY REFER TO

March 11, 1960

Dr. Joshua Lederberg Professor of Genetics Stanford University Medical Center Palo Alto, California

Dear Dr. Lederberg:

Your grant application titled "Cytochemical Study of Extraterrestrial Organisms," dated February 2, 1960, has been favorably considered and awaits administrative approval. Some negotiations between the NASA procurement and the Stanford University business offices are also necessary. It is anticipated that these steps will be carried on without further action on your part and that formal approval will be forthcoming.

Sincerely yours,

Clark T. Randt, M. D.

Clark T. Lander

Director

Office of Life Sciences

Space Administration 1520 H Street Northwest Washington 25, D. C.

Attn: Mr. William Willner, Procurement Officer

Gentlemen:

Enclosed herewith a budget for our proposed research on "Cytochemical Studies of Planetary Microorganisms." With the accompanying endorsement this constitutes a formal proposal from Stanford University. The scope of the intended work is as outlined in my letter of February 2, 1960 addressed to Dr. Homer Newell.

The indirect costs have been calculated at 15% of total direct costs. This figure now applies to the majority of federal research grants administered by Stanford University, e.g. from NIH and NSF. While we expect this percentage to be advanced to a higher and more realistic value of 20% or 25%, we do not wish to delay the initiation of this grant by negotiation for a higher figure than you would customarily offer at the present time.

We assume that in the absence of notice to the contrary, the grant will be administered in accord with grants from NSF and NIH, including such questions as the retention of title by Stanford University to purchased equipment, and the investigator's prerogative of rearranging the allocation of budget items in eccordance with the changing needs of the research program. However, expenditures for travel will not be increased over the budget without consultation with you. Please notify us of any specific limitations your agency may place on the disposition of funds under your research grant.

You will note that the starting date for the grant now reads April 1, 1960. This is mainly to allow for the placement of orders on which long delays for delivery are now quoted; consequently, your early approval to make commitments against the grant would be appreciated.

Yours sincerely.

Joshua Lederberg Professor and Executive BUDGET: PROPOSAL TO NASA FOR RESEARCH GRANT ON CYTOCHEMICAL STUDIES OF PLANETARY MICROORGANISMS

From: Laland Stanford, Jr., University, Stanford, California (Department of Genetics, Medical Center, Palo Alto, California)

Principal Investigator: Joshua Lederberg, Ph. D., Executive, Department of Genetics
For period April 1, 1960 to March 31, 1961

Professional and technical salaries (including DASI	
and retirement)	\$12,000
Equipment, purchase or construction	15,000
Supplies, expendable (laboratory, also Including office and reference)	3,000
Communications, secretarial and publication costs	1,500 =
Travel, for consultation on equipment and program planning	1,200
Subtotal and direct costs	32,700
Indirect costs, at 15% of direct costs	4,905
	37,605

*Exclusive of principal investigator

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STATUS OF EXOBIOLOGY PROGRAM

Some elements of the strategy of exobiological investigation have been discussed in previous reports (cf. Westex material as summarized by Space Science Board; Lederberg, J., 1960 "Exobiology - Experimental Approaches to Life Beyond the Earth," Science 132: 393-400, August 12). The first investigations of a new planetary habitat should certainly include a general survey at various magnifications for visual evidence of life; until the results of these are in, it is hard to judge what further steps should be taken in investigating the biology of possible larger organisms. Needless to say, if the first look does give evidence of Intelligent culture or of larger organisms, considerable effort would be devoted to characterizing these in later experiments. Meanwhile, a great deal can be planned for and done in microbiological investigation starting from first principles. There are many reasons to justify a strong emphasis on microbiology in planetary investigations just as there are for laboratory studies here. These include:

- 1) The greater likelihood of a successful result. In some habitats only micro-organisms might be present; on the other hand, it is difficult to conceive of a habitat which contained larger forms but did not contain micro-organisms.
- 2) <u>Ubiquity</u>. Even on a richly populated planet like ours, micro-organisms can be found in almost any-small sample of the atmosphere, the surface dust, or bodies of water. This is much less true of larger organisms.
- 3) <u>Metabolic diversity</u>. Micro-organisms should afford a wider variety of metabolic capabilities from which we might draw inferences about the comparative biochemistry of the planet.
- 4) <u>Ease of handling</u>. With any luck at all, at least some species of micro-organisms would be very much easier to cultivate and to contain for detailed experimental investigation.
- 5) Importance for terrestrial ecology. Both small and large organisms of external origin constitute potential hazard if inadvertently brought to the earth; one might anticipate much greater difficulties in removing micro-organisms and in controlling them if they should escape. Therefore, we should obtain as much information as possible about them in their natural habitat before programming experiments that involve the actual return of planetary samples to the earth.

How then might we procede to investigate the distribution of micro-organisms? We should be prepared to meet the expectations that:

- 1) A scarcity of moisture may result in a very sparse level of life.
- 2) The nutrition and biochemistry of the planetary organisms may differ quite markedly from terrestrial ones.

We must, therefore, stress the most efficient methods of detecting microorganisms and also those methods which do not require a too specific identification of earthly biochemical components. However, it is impossible to do a general experiment in the abstract; and concrete experiments necessarily involve some measured compromises on these principles. Further considerations in experimental design are the necessary limitations on the size of the pay load, the

J. Lederberg Stanford University 9/4/60 necessity for its automatic functioning, and the narrowness of the effective communication channel for returning the data to the earth. We should also keep in mind that apart from the existence of life on another planet, we are most deeply interested in a comparative study of its basic biochemical systems, and in particular, whether other types of compounds can take the place of nucleic acids and proteins of terrestrial organisms.

Proposed methods for exobiological study involve a combination of techniques and a systematic presentation is likely to be rather artificial. The following approaches may be kept in mind.

- l) Chemical composition and metabolic effect of the micro-organisms. This approach should be assiduously studied; the chief discouragement is the possible scarcity of material to analyze. However, in combination with, for example, controlled cultivation of the organisms, this approach will probably give us the most detailed information on the intrinsic biochemistry of the exo-organisms. Studies will be made to determine whether such characteristic substrates as ATP (adenosine-triphosphate) are sufficiently rapidly metabolized by small numbers of organisms to warrant further developments along this line.
- 2) Cultivation of the organisms on prepared media. This is the habitual technique of the microbiologist and will doubtless have very high priority when we can make firmer decisions on exo-microbial nutrition. Where water is a limiting factor in a planetary biology, this may prove to be the most important nutrient. If the communication channel is so narrow that only a few bits are available, an efficient black box design, incorporating a test of microbial growth, and giving a simple yes/no answer might be desired. However, in view of our uncertainties as to the optimal nutrients, it would be wise to test a variety of alternative media. Such a system might be miniaturized by culturing the organisms on moistened spots on a moving tape rather than in bulk culture. The likelihood that the communication channel, though still restricted, might be more ample suggests a more detailed study of the samples. The two most promising procedures appear to be microscopy and spectrophotometry, or some combination of the two.
- 3) Direct optical examination of the organisms (microscopy and spectrophotometry). These methods involve the measurement of light intensity as a function of position (i.e., the image of a particle) or of the spectral wave length (i.e., its absorption spectrum). If the technical problems can be surmounted, the combination of form and spectral data would give a most powerful method of biological analysis.
- a) Type of microscope. Most living cells, including bacteria, are essentially transparent to the customarily visible wave lengths and must be processed in particular ways in order to achieve useful contrast. The traditional way of doing this in the laboratory is by staining, a procedure which can be used for a considerable degree of chemical specification, but which adds to the mechanical complications. Two principal possibilities are left for the observation of unstained objects: microscopy in the ultra-violet at 2600-2800 Å and phase contrast microscopy. The advantage of ultra-violet microscopy would be the relative chemical selectivity at different wave lengths, for example, the very high absorption by nucleic acids at around 2600 Å. However, to take full advantage of this selectivity, additional information on the absorption at one or more other wave lengths would be desirable. Likewise, with phase contrast microscopy, the



greatest information would be obtained from a system which also discriminated between particles which were opaque vs. transparent in ordinary transmission microscopy. Therefore, the efficient use of either UV or phase contrast microscopy suggests the development of some method of color discrimination. This does not necessitate the transmission of full color data, but rather the interposition of a "filter logic" to pass only those signals that fulfill stated criteria (i.e., transparent in visible and opaque in UV or phase contrast). Such finesse should be more appropriate for electronic than photographic recording of the signals, and our preliminary efforts have been directed to evaluating a UV microscope-vidicon chain. Recent work on image-intensifying UV camera tubes (The Westinghouse Ebicon being constructed for the orbital UV telescope) may be extremely useful; other arguments favor the adoption of flying-spot technique into the spectrophotometric-microscope system.

- b) Preliminary data reduction in situ. The limited communication channel suggests the most strenuous efforts to eliminate irrelevancy and unwanted redundancy from the transmissions. These arise from at least two sources within the object being looked at:
- 1) A probable abundance of biologically uninteresting material, especially mineral particles, among which it may be difficult to discriminate cells and
- 2) High point-to-point correlations in the object being looked at - for example, under phase contrast, bacteria often resemble homogeneous elipsoids.

The waste resulting from the second factor might be minimized by differential and edge-contrast discrimination techniques; the discrimination of color, and perhaps also of form, might be used to monitor a decision as to which elements were worth transmitting. (Needless to say, at least a few unfiltered pictures would be desirable also.) Waste from the first source could be minimized by methods for the concentration of living material and the rejection of other extraneous particles. The <u>cultivation</u> of the organisms, if this succeeds, would be an admirable way to do this. However, in the expectation of possible failure, thought should be given to other methods for the separation of cells from various kinds of debris.

The problems of data reduction summarized here have some analogy to the problems involved in the effective use of satellite reconnaissance (Samos) (cf., Katz's review in <u>Astronautics</u>, July, 1960.).

4) Collection of samples. So far the least attention has been given this problem, perhaps in the anticipation of productive developments in other fields. For example, the auger and corer that have been proposed for the lunar round-trip should be quite applicable to the present purposes except that the specimens would be processed within the payload rather than being returned. In addition, the choice of procedures for collection would depend on the types of examination that were adopted. At the present time, three alternatives are evident.

a) The collection of atmospheric dust for direct microscopic examination on adhesive tapes - the flypaper proposal. b) The collection and concentration of larger amounts of atmospheric dust by impaction or filtration - methods now in standard use in aerial microbiology. c) The soil sampler.

The first method might be applicable for direct inspection and also cultivation on the tapes themselves. However, if the payload weight permits, it would be safer to collect a larger sample and enrich it by the flotation of cells in a dense medium as described further.

5) Present judgments. At the present time, I would suggest a program with the following objectives. a) The collection of from 10-100 grams of dust or soil. b) The fractionation of the sample by flotation and c) direct examination of the organic fraction by 1) phase contrast/transmission dual microscopy and 2) microspectrophotometry between 2400 and 8000 Å, either on single particles or on cleared samples of the organic fraction. c) Data reduction and programming to transmit only the more likely observations. Part of the sample should also be cultivated on a nutrient tape and then also examined in like fashion.

It will be difficult to make more refined choices and decisions until the hardware has been designed and constructed to the point where it can be tried out extensively on a larger variety of terrestrial samples. One of our necessary and most important functions long before space flight will be the calibration of these procedures and the search for the kinds of artifacts that must be avoided.

6) Further refinements and later experiments. With appropriate support and cooperation of industrial organizations a workable system incorporating some or all of the above elements should be ready in ample time for Saturn flights to Mars circa 1965. If time still permits, additional refinements can be added for the first missions and must be planned for succeeding ones. These might include a) More complete cytochemical tests of observed particles, in particular for DNA and for proteins. It should be possible to adjoin these tests to the microscopic observation. b) Command vs. automatic control of the instrumentation to allow for unexpected developments at the target. c) Larger scale enrichments for Martian organisms. These might depend on good guesses or acquired information on the chemistry of the Martian surface and on the physiology of the Martian organisms, and could then expand this knowledge.

Report to the National Aeronautics and Space Administration
"Cytochemical Studies of Planetary Microorganisms - Explorations in Exobiology"

NsG 81-60

April 1, 1960 - March 31, 1961

Department of Genetics School of Medicine Stanford University Palo Alto, California

This report covers operations on a preliminary grant. This has since been renewed and extended (NsG 81-60 (S1)) to read from April 1, 1961 - March 31, 1964.

Joshua Lederberg Principal Investigator The first year's operations were devoted primarily to:

- defining the experimental role that a laboratory at Stanford might play in exobiological work
- 11. assembling basic instrumentation
- III. enlisting and training personnel
 - other agents of NASA in validating experiments in exobiology, and are continuing to do so. (2) The main lines of work in progress include see the numbered exhibits for details.
 - (a) The design and construction of a fast scanning microspectrophotometer. A digitized camera control for variable rate and raster pattern has been designed as a necessary element for adapting the U-V vidicon as a sensor for spectrometry. It is now under construction.
 - (b) Spectral analysis of microorganisms and other soil particles. This has been started, pending the completion of (a), with improvised, slow designs and with the cooperation of Professor Caspersson in Stockholm. They have so far verified that various microorganisms have characteristic absorption maxima in the region of 260-280 mµ, a property so far not shared by other soil particles. However, rare earth samples which might be expected to be the likeliest source of spectral mimics have not yet been studied.
 - (c) Sensitive enzyme tests for use in the multivator (2). The assay of phosphatase by the release of (colored) nitrophenol from nitrophenyl phosphate has been used as a point of departure.

Extensive trials on soils have shown the feasibility of detecting activities from soil samples of about 10 mg in one hour. This is at least ten times too <u>insensitive</u> for feasible application in the multivator. However, comparable <u>fluorescence</u> assays should be several orders of magnitude more sensitive, and await the synthesis of some new substrates, as has been arranged for. This is part of the multivator project in cooperation with JPL.

(d) Concentration of microbes in dense solutions for microscopy. (4) This has been incorporated into an "RFP" from JPL for further industrial design study on a detection system.

11. Equipment

We have been assembling and modifying the necessary equipment for spectral .

and fluorescence measurements. At present we have the basic tools (light sources, monochromator: recording spectrophotometer, closed circuit TV chain, oscilloscopes, amplifiers and other electronic gear), while the more refined instruments are being designed and built.

III. Personnel

The research team is crucial to the long range success of this venture, and it has taken a long time to find the appropriate talent, as we have had to build a new group of rather nonconventional makeup. With Dr. Levinthal and Mr. Horn we now have an excellent nucleus. The scientific staff now includes:

Professor Joshua Lederberg*, Principal Investigator

Dr. Elliott C. Levinthal*, Research Physicist, Technical Manager

Mr. Harrison Horn*, Electrical Engineer

Miss Haruko Nagaishi (B.A.) and Mr. Elliot Packer (B.A.), Microbiologists.

Another experienced engineer, Mr. L. Lee Hundley is joining us this summer.

We are looking for an experienced microbiologist - biochemist as a research associate (Ph.D.) level to round out the group.

(* see curriculum vitae (1)).

This is offered as an interim report, as the laboratory is just gaining momentum, and important factual contributions will await the assembly of instruments now in the design - early construction state. Some experimental results are summarized in the attached exhibits:

- 1. Curriculum Vitae
- 2. Explorations in Exobiology, Cytochemical Studies of Planetary Microorganisms A program of research at Stanford University.
- 3. Multivator. Proposal for Mariner B Experiment Capsule
- Cytochemical Studies of Planetary Microorganisms Explorations in Exobiology. A research proposal to the National Aeronautics and Space Administration.

PROGRESS REPORT ON WORK AT STANFORD ON EXOBIOLOGY

This will be rather brief although we have had notification of support from NASA since about mid-May. Procurement has been slow and components have only recently been assembled so that we can begin to check out a system of UV Vidicon microscopy. The new Zeiss achromatic UV objectives have just recently arrived and these appear to be far superior to the previously available mirror objectives and catadioptric lenses that were previously available. We have assembled a system for microscopy at 2537 A using a low pressure mercury source, interference and chemical absorption filters (neither of them very satisfactory), the Zeiss objectives, and a television chain incorporating a UV-sensitive Vidicon tube (two samples of the RCA Vidicon and one from ENI in England). The latter seems to give a superior picture with better resolution, short lag and, probably, higher sensitivity. However, both tubes, although designated as UV-sensitive, are rather disappointing in their performance at <3000 A and probably have not been designed primarily for such an application. They would be relatively satisfactory if we could operate at considerably higher intensities of monochromatic UV illumination but this is difficult to obtain in any event and should probably be avoided because of its potential damage to viability and to organic materials. The present microscopic arrangement is, however, already quite satisfactory for photographic recording. The answer to our present problems might lie a) in improvement of the light source and filters, or perhaps better the incorporation of a simple monochromator and b) the use of the much more sensitive image-intensifying UV tube, the Ebicon. In any case, this development should be just the first step of a system in which images can be compared as between the ultra-violet and the visible. (The flying-spot microscope design is capable of much higher intrinsic sensitivity than the Vidicon image tube, since a photomultiplier can be used as the detector; however, the Ebicon may give a comparable result.)

As an alternative to the use of ultra-violet for the visualization of transparent objects, the new technically much simpler phase contrast optics might be employed. Again these would be especially advantageous if a simultaneous recording could be made of the <u>form</u> of a particle in phase contrast and its <u>transparency</u> by transmission optics. This might be converted into color rather readily by the use of narrow pass filter elements in place of opaque diaphragms in the phase optics ("color phase contrast").

Much more information can then be obtained from UV optical methods by obtaining an absorption spectrum, particularly in the range between 2400 and 3000 A. This UV information would be parallel to the image formation, and both sets of data might be collected with the same optical system, the scan alternating between wave length and position of the beam.

As far as I can determine, UV spectra of individual bacteria obtained in such a fashion have not be published, but this partly reflects the limitations of the microscope optics available until now. The Zeiss achromatic objectives were designed for just such analytical purposes at the instance of Caspersson in Stockholm. He believes that it would be technically feasible to obtain such spectra, and has agreed to run some representative cultures on his own instrument during the next few months. Serious problems, e.g. correction of scattering

J. Lederberg Stanford University 9/4/60 losses, and especially the installation of satisfactory light sources must be dealt with, but this approach is potentially the most powerful that could be devised for the analysis of micro-organisms by remote technique. If the technical development is successful, particles could be scanned for their spectra, and this information then used to monitor the transmission of selected images which consume more communication time.

Some of the most encouraging results obtained so far have been in the fractionation of organic material from soils. After a considerable search for suitable solutions of adequate density, most of which were for one reason or another rather unsatisfactory, we tested "Ludox" at the suggestion of Kopac from NYU. Ludox is a colloidal suspension of silica in water and can be made to a density as high as 1.20, which is more than adequate for the flotation of living cells, while almost all minerals still sediment in it. The silica being chemically almost inert and having no appreciable osmotic effect is relatively harmless to the cells and leaves them quite viable. A number of types of soil sample have been fractionated by flotation in Ludox and the method has proven to be startingly efficient in the detection of micro-organisms even from rather unpromising sources, for example, volcanic sand collected at 14,000 feet from Mt. Popocatapetl: old dried mud from a core dredged from the Mariana Trench, and dust that had settled on the roof of a car and exposed for appreciable time to California sunshine. In each case, the supernatant fraction was highly enriched for bacteria and these could readily be seen at the very first glance through the microscope. The same samples had only a marginally detectable microbial population when examined directly. Samples from richer soils also displayed an abundance of other kinds of organisms, with relatively little interference from residual adherent soil particles. We are now seeking even more marginal samples to see whether there is any likely sample from the earth's surface in which we would not readily detect microbial life by this method. The flotation-separation is facilitated by spinning the samples in a centrifuge and larger samples could be efficiently processed by a continuous flow sedimentation. However, smaller samples will sediment if merely allowed to stand several hours. We are now designing a cell which will allow for microscopic observation with no handling after the sample is introduced into the cell. The relatively low density of living particles, having an aqueous base, should be a fairly general principle for the preliminary separation of them from mineral fragments. More refined criteria are of course needed for a final conclusion.

(This method of separation promises to have great value as a general laboratory procedure and may have practical applications in other areas of medicine.)

The basic feasibility of the proposal to grow bacteria on permeable tapes has been verified; flattened tubes of cellophane dialysis tubing were impregnated with nutrient broth and would then very satisfactorily support the growth of various bacteria and fungi. These were then very informative when examined under the microscope. The chief problem appears to be the loss of moisture by evaporation so that the tapes will have to be maintained in a closed humidified chamber. In view of the long transit time of the space craft, the tapes may have to be flown dry and filled with water only at the time of the experiment; alternatively nutrients may be prevented from diffusing from one site to another by mounting the

culture spots as blisters on an impermeable, e.g. quartz, ribbon. We have not succeeded in obtaining quartz ribbons as might be extremely useful as tapes for the indicated purpose but they should not be too difficult to fabricate. More mechanical skill and ingenuity than we can readily muster should be centered on the production of prototype hardware along these lines for testing and improvement.

Recommendations

Our experience to date suggests that the basic strategic principles that had been formerly enunciated are sound and should be exploited further as the basis of exobiological detection. However, as we have now reached the stage where considerable new hardware, both electronic and mechanical, must be designed and built, it is essential that we have the cooperation of an engineering facility. Needless to say, we would be very happy to maintain a close continuing contact with such a facility and in particular to assess the results for their biological usefulness.

In view of the pressure of vehicle schedules, it would be foolhardy to delay the development of prototype hardware for the exploration of every worthwhile accessory. New technical advances and new insights into scientific goals would lead to indefinite postponement of any actual experiment.

Enough information is now at hand to justify the construction of the basic mechanism of an automatic microscope. This should have provision for the transport of specimens, focus, photoelectric conversion, color discrimination, signal reduction and telemetry. The first version might be designed to use color phase contrast, which is technically simpler than UV microscopy. This basic instrument would be a valuable tool even if time did not permit the addition of the accessories to improve its sensitivity and precision. Concurrently, design work should proceed on the technique of collection of samples, and on their centrifugal fractionation, to furnish the input to the detector system. This much of the system should lie within the reach of present art. Study work and preliminary trial are also required for the microspectrophotometer accessory to the microscope, which does pose serious technical problems, especially the provision of a suitable light source. A bench model should be constructed for tests on terrestrial samples as a preliminary to its adaptation to the system for spaceflight. By concurrent work on the particularized problems thus indicated, we can be sure of having the best instrument that we had the means to develop in time for schedules of planetary flight. There are cogent reasons besides the relative scientific impatience of biologists why such instrumentation should accompany the earliest planetary explorations. Laboratory work in biology does not now have the benefit of a well-established tradition of development of instrumentation. Much more than in the physical sciences, spaceflight research in biology will have to develop new instruments for which only the principles, and not their technical realization, are already current in laboratory practice.

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DETECTION SYSTEM FOR EXOBIOLOGY

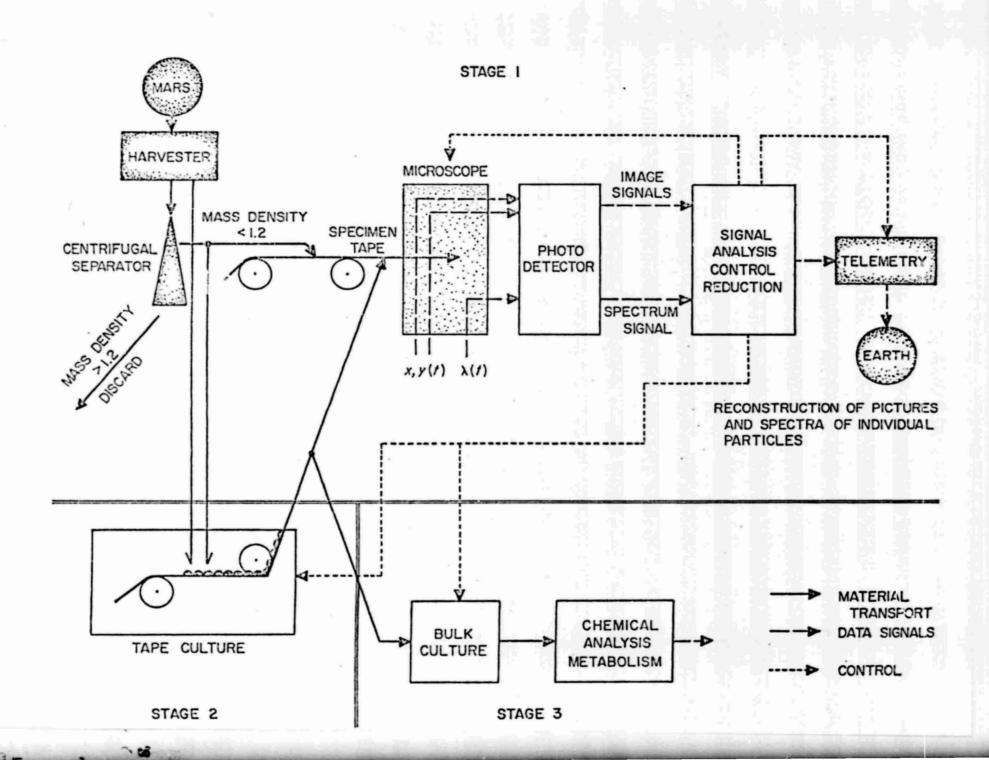
Prototype for Martian soft landing

- HARVESTER. To collect 100 grams of soil or dust. ("vacuum cleaner"; air impacter; air filter)
- WINNOWER (Centrifugal separator). To separate light and heavy fractions of the soil by sedimentation/flotation in dense liquid (e.g. Ludox d = 1.20). Heavy fraction is discarded (d > 1.2); light fraction is transported to
- 3. SPECIMEN TAPE which carries specimens into
- 4a. MICROSCOPE, the input light beams of which are modulated for
 - a. position coordinates, to give an <u>image</u> signal, at two wavelengths in color phase contrast
 - b. wavelength, to give a spectrum signal, constituting a
- 4b. MICROSPECTROPHOTOMETER. (Specs. range 2400 4400 Å; resolution 50 Å; 8 intensity levels)
- 5. PHOTODETECTORS convert the optical to electrical signals which are the input to
- SIGNAL analysis system. (The aim of this system is to discriminate those
 particles which have some color in the ultraviolet, and void those particles
 which show monotonous absorption or scattering.)
 - a. Filter logic on 4a. signals, inverting the phase from opaque objects to delete their signals

----and test as alternative to a.----

- Recognition of 'interesting' spectra, for control of further processing
- c. Reduction of image information from 'interesting' particles for efficient telemetry of picture of individual particle and associated spectrum.
- 7. TELEMETRY to EARTH
- 8. RECEPTION of signals and RECONSTRUCTION of pictures of individual particles and associated spectra.

THROUGHOUT THE DEVELOPMENT, THE SYSTEM MUST BE CALIBRATED AGAINST TERRESTRIAL SOILS IN ORDER TO VERIFY AND IMPROVE THE DESIGN AND TO ACCUMULATE EXPERIENCE FOR THE INTERPRETATION OF THE REDUCED DATA.



TV Microscope May Discover Life on Other Planets

If in the next few years man discovers evidence that there is life beyond the Earth, he may ove his hard-won knowledge to a planetary television station now being designed at Stanford Medical Center.

The designer is Dr. Joshua Lederberg, executive head of the Department of Genetics in the Medical School and a Nobel laureate.

What does Dr. Lederberg hope his instruments will find on another planet? Not men or monsters of space fiction. Not monkeys or even fish in the sea. All these would be too hard to find--if they exist at all.

Instead, he proposes to look for the sort of life that would be found "in any drop of water, pinch of soil or gust of wind"--mi-croscopic life of the sort he has studied in great detail for many years.

"Given a limited sample for study, microbiological analysis will certainly give the most reliable diagnosis for the presence of life anywhere on the planet," he says. "We cannot imagine another world lacking microbes if it has any form of life at all."

Accordingly, the Stanford geneticist has been at work for more than a year trying to plan the most suitable system for detecting and studying these tiny specks of life. Support for the research has come from the Rockefeller Foundation and the National Aeronautics and Space Administration. At the heart of his plan is a microscope, but not the Earth-bound kind.

This microscope will utilize ultraviolet



DR. JOSHUA LEDERBERG, who is designing instruments to detect planetary life.

light, which will distinguish microbes from mineral particles. It will be fed samples by means of a sticky tape. In turn, it will relay the visual information back to Earth by television.

The picture it shows might be the most important TV program for all time if it answered one of these questions: Is there life on another planet? Does it have the same chemical basis as our life? Are there any clues to the origin of life on Earth?

The televised image should also give some insight into the possible hazards of contamination of the Earth from planetary organisms. The most dramatic hazard would

be the introduction of a new disease, imperilling human health.

"Even the remotest risk of pandemic disease....must dictate a stringent embargo on the premature return of planetary samples, or craft that might inadvertently carry them."

Mars is the likeliest target for the study of "exobiology," Lederberg's own term for extra-terrestrial life, but he predicts that it will be from five to eight years longer before the microscope is ready to soar off into space.

"Exobiology is no more fantastic than is the realization of space travel itself," he says. "We have a grave responsibility to explore its implications for science and for human welfare with our best scientific insights and knowledge."



CAMERAS AND LIGHTS filled the lab when NASA made a movie for Congress about Dr. Lederberg's studies.

EXPLORATIONS IN EXOBIOLOGY

CYTOCHEMICAL STUDIES OF PLANETARY HICROORGANISMS

A Program of Research at Stanford University

Joshua Lederberg
Professor of Genetics
Stanford University Medical School
Palo Alto, California

Since December 1959 with the help of preliminary support from the Rockefeller Foundation, and since April 1, 1960, with the extensive support of the U. S. National Aeronautics and Space Administration (NASA), a laboratory has been established in the Department of Genetics at Stanford University in the new field of exobiology. Its aim is to lay the groundwork for the experimental study of the existence and characteristics of life that may have evolved on other planets of the solar system.

The NASA's program for space science calls for the flight of probes to the vicinity of the nearby planets in the interval 1962-1964 and for the landing of experimental spacecraft on planetary surfaces within the subsequent few years. These spacecraft will have the capacity to carry and operate a variety of scientific Instruments whose information can then be telemetered back to the earth. The actual retrieval of samples from planetary surfaces and their return to the earth for detailed analysis is envisaged in the long-range program of space exploration. However, this cannot readily be achieved with vehicles based on present methods of propulsion and will, therefore, probably not be accomplished before the next decade. The initial search for evidence of planetary life, beyond the indirect evidence from telescopic observation, therefore depends on instruments that can function automatically and transmit data by radio from the target planet.

The interest of this laboratory is focussed on the detection of microorganisms, with special emphasis on the detection of nucleic acids and of
proteins. We believe that the most important aspect of the study of
extraterrestrial life is the possibility of learning in how many different
ways life can evolve. The nucleic acids and proteins are the essential
common features of terrestrial life. Whether forms of life could exist
based on other substances or related analogues of these substances, is
one of the most fundamental challenges to biological theory.

The laboratory's mission includes both engineering and scientific objectives, though both are directed to the ultimate acquisition of fundamental data. The first is the development of advanced instrumentation, adaptable to spaceflight, capable of making the necessary determinations on samples of a planetary surface. The second line of investigation is the study of optical, electrical, and other physical and chemical properties of microorganisms that might be relevant to the general definition of life and to characterizing such organisms in a planetary environment. The challenge of exobiology has required a re-orientation of our outlook on the means of recognizing and detecting life; this has uncovered a number of elementary but potentially quite important, problems both in the area of instrumentation and of the properties and functions of organisms.

We are obligated to make a continuing, critical review of the types of measurements that are best suited to the unique opportunities and constraints of actual space research. This review, which is carried out in concert with a number of investigators at several universities reporting to the National Academy of Sciences and to the NASA, is leading to a continual re-definition of the goals of space biology with higher and higher precision. 1,2 Meanwhile, in order to meet the timetable of vehicle development and flight, the best choices must be made for the development of practical instruments.

Several approaches are currently under study:

- (1) The concentration of living organisms from soil by flotation methods. Since organisms generally have a high content of water, their specific gravity is rarely very different from 1.0. Most minerals, on the other hand, are considerably denser. Organisms can therefore be efficiently extracted from soils in which they represent but a small part by weight by the technique of flotation in a medium of intermediate specific gravity. In such a medium, the mineral components, in general, will be sedimented whereas the organisms will float to the top.
- (2) Microscopic examination of particles. Historically most of our perception of the widespread occurrence of microbial life on the earth has stemmed from the use of the microscope starting with Leeuwenhoek in 1674. In many cases, microorganisms can be recognized by their characteristic form and organization. However, such observations would still give us only a limited certainty that we were dealing with a living particle and an equally limited knowledge of its chemical composition.
- (3) Spectral analysis. The information on a particle under microscopic observation would be enhanced if it also included the principal optical properties of the specimen. Such properties are, for example, the absorption spectrum (in effect, the color) particularly in the ultraviolet range, refractive Index and polarization properties and related measurements. Several of these measurements can be made with equipment that is already in widespread use. For further development work, we are laying special emphasis on the design and construction of a rapid scanning microspectrophotometer. This device should have the capacity to delineate the absorption spectrum of a particle in the ultraviolet. If it can be constructed to operate with sufficient speed and precision, this instrument could be used for the rapid search of a large sample for those particles that have characteristic absorption spectra such as typify the organic constituents of terrestrial organisms. High precision microspectrophotometers have already been constructed in several laboratories, especially that of Professor T. Caspersson at the Karolinska Institute In Stockholm. As measurements made in these laboratories have shown, the most characteristic spectral feature of microorganisms is a specific absorption in the ultraviolet at 260 nm (= 2,600 Å). This absorption is attributed to the presence of relatively large amounts of ribonucleic acid. Relatively little is known about the optical properties of other particles likely to be found in soils and a review of such material is an essential part of the program. It is visualized that the rapid scanning, search device would have the capacity to recognize spectra that have "interesting" features according to the criteria that can be preset for this. The instrument should, then, have the capacity to select those particles whose spectra indicate them to be of the greatest interest for more detailed observation. In this way, it should be possible to minimize the amount of power required for the radio transmission of the Indicative Information.

- Cytochemical procedures. Still more precise characterization of the chemical composition of microscopic particles can be obtained by an extension of present methods used in cytochemical research. For example, the recognition of ribonucleic acid can be made virtually certain by the action of the specific enzyme ribonuclease. This could be ascertained by the disappearance of the characteristic ultraviolet absorption of a particle In the presence of this enzyme or by the application of other staining techniques that give differential colors observable in the visible wavelengths.
- (5) Optical study of bulk samples. The application of these techniques on a microscopic basis requires a certain optimism for the development of Instruments that will have a sensitivity and precision adequate for the task. Such Instruments, even if successfully constructed, may also require a time for their development, and assignments of weight and power that cannot be matched for earlier spaceflight missions to the planets. To face this contingency, parallel techniques should be developed that can be applied with somewhat less precision to larger samples of material examined in bulk. This approach will require substantial improvement in the general technique of optical and spectral examination of impure samples.
- (6) Enzyme analysis. Another line of evidence for the existence of living organisms would rely upon the detection of various specific enzymes manifest in or extracted from bulk samples of a soil. Ultra-sensitive techniques for enzyme detection have been developed (by Dr. Boris Rotman, currently in this department) that have the capability under favorable circumstances of detecting even single enzyme molecules. Such methods are being surveyed for their application to the detection of enzymes such as phosphatase, ATPase, ribonuclease and deoxyribonuclease, whose presence would be an important lead to the nature of the living system.
- (7) Artificial cultivation. One of the most decisive approaches to the detection and characterization of extraterrestrial life would be its artificial cultivation in the same sense that we readily cultivate microorganisms in the test tube in our laboratories here. One of the chief limitations to this approach is our lack of detailed information as to the chemical composition of planetary surfaces - information which is vitally important in designing the appropriate culture media on which such organisms would be expected to thrive. Nevertheless, considerable effort should be given to the design of suitable devices in which the growth of implanted microorganisms could be detected when reasonable choices for the appropriate culture medium are possible.

Three celestial bodies, the moon, the planet Venus and the planet Mars, are within the range of the spacecraft of the present decade. The presence of life beyond the earth anywhere in the solar system is a problematical issue and many a priori arguments can be raised that would discourage our efforts to search for such life. However, our information on the conditions on these bodies is far from exact and it is also difficult to be sure of the versatility that life may have evolved in meeting the problems of existence in other environments. In any case, the importance of the discovery of other forms of life is so great as to obligate us to make the most strenuous efforts to ensure that no reasonable possibility has been overlooked. Within these limitations, the following assessment of the celestial bodies can be made.

- The moon. The virtual absence of any atmosphere on the moon also precludes the presence of significant amounts of liquid water or other volatile materials on the lunar surface. We can therefore be reasonably sure that active life is impossible on the moon. However, the moon is a repository of large numbers of meteoritic particles that collide with Its surface, and it would be of the utmost Importance to learn whether such particles ever carry traces of organisms as might be transmitted from the earth or from elsewhere in the solar system or beyond. For this reason alone, once the basic physical and chemical properties of the moon's surface have been adequately investigated, we may wish to consider the means and possibility of making a comprehensive search for such traces of life from elsewhere. In addition, it can be argued that while we can exclude any form of active life on the exposed surface of the moon, we know too little of the subsurface strata to make any categorical statements as to the environment and what may have evolved there. Again, we are hardly in a position to assess this challenge, much less define the means of meeting it until we have secured much more analytical information from chemical and physical studies.
- (B) Venus. The clouds that surround this planet have virtually prevented useful telescopic observation of the planetary surface. The first task of spacecraft exploration of Venus will then be to penetrate below the clouds. Several lines of evidence based on the measurement of the intensity of radio frequency radiation from the planet suggests that the surface may have a very high temperature, so high as to preclude the persistence of living forms. The verification of surface temperatures is therefore a prerequisite to further biological study of this planet.
- Mars. Of the other bodies of the solar system, Mars presents the highest probability of sustaining forms of life comparable in any way to those we recognize on the earth. Even at that, this probability is a highly uncertain one. Indirect measurements suggest that the temperature of Venus, while slightly cooler than that of the earth, would still be reasonably compatible with simple forms of life. However, the level of oxygen in the Mars atmosphere is so low that it has not yet been detected by telescopic means. Even more significant, while there is sufficient moisture on Mars to result in the accumulation of polar caps of frost, present estimates would indicate that Mars as a whole, is far more arid than the dryest parts of the earth's surface. If this is generally true, the extensive development of Martian life forms would necessitate some local "meteorological" exceptions to the general level of low humidity together with the evolution of special mechanisms for the accumulation of water within the supposed organisms. At the very least, the procedures for attempting to diagnose the presence of living particles on Mars must take account of these severe limitations. On the other hand, at least a hint of the presence of Martian life has been obtained through telescopic observations: the visible dark areas on the planetary surface under to striking seasonal changes Furthermore, they show optical absorption features, in infrared wavelengths, that are highly indicative of the presence of appreciable amounts of carbon-containing compounds such as those from which life on the earth is constructed.

For these reasons, the exobiology program in this laboratory and elsewhere is directed primarily to the question of life on Mars, although the application of similar experimental techniques to other celestial bodies is also in mind. In succeeding decades, our space vehicles may be able to penetrate further - to the major planets, especially Jupiter, and these will represent special problems in the possibilities of biological investigation. The environmental conditions on these planets - the high concentration of hydrogen, carbon, nitrogen and oxygen on the one hand, and the very low temperatures and high pressures on the other, suggest that the major planets will be of extraordinary interest from the standpoint of primitive organic chamical mechanisms but that any forms of life that might have evolved there, would have to be substantially different from those that we would recognize here. In any case, the approach to these planets from both a vahicular and instrumental standpoint poses immense problems of so different a magnitude from those we face now, that they may well be deferred until we have a better appreciation of the properties of the nearer planets.

REFERENCES

- 1. The NASA programs are outlined from time to time in hearings of the House Committee on Science and Astronautics and the Senate Committee on Aeronautical and Space Sciences, and in the semiannual reports of the NASA to the Congress. A convenient recent summary of NASA programs has been published as: "NASA-Industry Program Plans Conference", 1960, available from the Superintendent of Documents. See page 68.
- (2) See "Science in Space", Report of the Space Science Board, National Academy of Sciences, Washington, D.C., 1960 and to be published as a single volume by McGraw Hill, New York, 1961. Chapter 9, "The Biological Sciences and Space Research," has also been published as: Lederberg, J., 1960. "Approaches to life beyond the Earth." Science 132: 393-400.
- (3) Sinton, William M., 1959. "Further evidence of vegetation on Mars." Science 130: 1234-1237.

Department of Genetics School of Medicine November 4, 1960
INSTRUMENTATION RESEARCH
LABORATORY

Dr. Homer Newell
Chairman, Planetary and
Interplanetary Science Committee
Headquarters, National Aeronautics and
Space Administration
1520 H Street Northwest
Washington 25, D. C.

Dear Homer:

The accompanying remarks have been contrived with the great help of a session of the Space Science Board Exobiology Committee on October 29th and I am submitting them now as partial response to your request for comment on the Short Range Program. It should be read as a postscript to the item marked 8-S which Dr. Young should be delivering to you.

At the last session of the Committee, you stressed the importance of the lead times necessary to accomodate scientific experiments on the appropriate space craft and you particularly indicated that these times would become longer the more capable and the more complex the vehicle. In these circumstances, it seems to me an urgent necessity to proceed as rapidly as possible with the development of the scientific hardware, and especially the simpler biological experiments that have not yet been checked out in space craft environments. At the present time, it may be possible to meet current schedules with carefully thought out and well tried designs but it is obvious that we have no comfortable leeway in time and the advantage we now have may be dissipated if we are not able to move promptly on the actual instrumentation. Please-let me know the most effective channels to which these remarks, as coming from a University scientist outside the administration, may be directed. I need hardly point out that the Space Science Board has repeatedly urged the same views. The two basic exobiology instruments that are ready for implementation at the present time are (1) a culture device (the "multivator") which is essentially a test tube rack in which various nutrient

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media can be accomodated and changes noted. The same basic device would also be apropos for simple chemical reactions, and (2) a microscope which can be considered as one of the lens options available to any photographic system. The basic ideas behind these instruments are extremely simple and one foresees little difficulty in their implementation. However, until they are constructed so as to suit space craft requirements, we cannot be sure that considerable development work may be necessary to prepare any bugs that may develop. Meanwhile, in laboratories like my own, further work can be done with an aim to improving the sophistication of these instruments and making them more incisive for specific experimental goals. For example, we hope to investigate the potential use of microscopic spectrophotometry for the characterization of individual particles. If these efforts show the feasibility of such an instrument, it can be built on to the basic microscope just described. However, it would be rather risky to stake the early success of a program in exobiological exploration on the outcome of these trials and the basic microscope alone can be expected to furnish a substantial yield of useful data.

It should be obvious that in the course of time, our plans are bound to shift. Some programs will slip and the schedules have to be set back; on the other hand, we may suddenly observe unexpected opportunities. Again, in order to cope with the eventualities in a reasonable and effective way, it is absolutely essential that we have ready a repertoire of basic experimental devices for use in biological as well as in physical observation.

Looking forward to seeing you at the meeting on November 15,

Yours sincerely,

/s/ Joshua Lederberg Professor of Genetics Interplanetary Subcommittee
National Aeronautics and Space Administration Oct. 31, 1960

Further Comments on Mars 1964 (Exobiology)

1. Previous comments (8-8) on Mars 1964 were predicated on the tentative assumption that the capsule could maintain direct communication to earth at a very limited rate but for many days or weeks. This program would have made it impossible to conduct any very detailed experiments involving high rates of information transfer but the long duration of contact would have encouraged longer term culture experiments.

More recent information on the current analysis of the situation suggests a rather different program involving a relay to the passing bus. On this basis, a channel of about 1 KC might be available for about an hour, this decaying to 1 cycle in about a day. Such a design puts a very different complexion on the mission, primarily insofar as it opens up the opportunity for a substantial number of photographs to be transmitted during the early stages of the mission. Such photographs can be taken at very long range from the passing bus but the opportunity for close-ups is much more exciting. Such close-ups should be invaluable in setting the scene for later experimentation by delineating the overall habitat and its variability. In addition, some specific aspects of the geological history of the planet, and even (as a remote possibility) the presence of larger recognizable forms of life might be apparent.

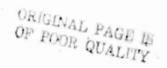
If something of the order of 25-50 photographs could be obtained during the first hour or two, we might wish to reconcile two conflicting aims: (1) to obtain views as close up as possible and (2) to reconnoitre over as large an area as possible in order to secure a broader perspective of the planet. The first objective might seem to entail a definitive landing, while the second would be better met by a slow descent utilizing an air foil or a balloon.

After some debate as between these alternatives, a third possibility has been brought up for discussion and is submitted with a view to eliciting a critical analysis of its feasibility. This would involve a semi-buoyant structure which might graze the ground and then be lifted again either by wind action or by programmed release of buoyant gas.

An additional advantage to this design is that it may afford a possibility of solving the rather perplexing problem of how best to collect samples of the ground surface with the least complication in gadgetry. The grazing balloon could be expected to scrape loose material from the surface into small drag buckets, thus utilizing wind energy in place of more complex schemes of mechanical actuation.

Until the capabilities and limitations of the system have been explored, it would be difficult to enumerate all of the most desirable experiments. The following are of evident interest:

- 1. Vidicon photography at various focal lengths. For pictures from altitude, a video-signal-controlled Zoomar could even serve as a range altimeter. The close-ups should include magnifying lenses that could give down to 1 mm resolution with the hope of identifying "vegetation".
- 2. Vidicon microscopy in effect, another objective lens for the Vidicon system. At minimum, this lens could look at dust collected on a sticky tape mounted at the focal plane. Resolution to 10 microns should not be too difficult to obtain and 1 micron should be attempted. The possibility of mounting a small illuminating lamp (drain 100 milliwatts) within the objective deserves consideration. If this is feasible the microscope need not weigh more than a few ounces all together.
 - 3. A radiobolometer.
- 4. A contact thermometer for the calibration of 3. at points of contact.
- 5. A microphore can serve as an impact sensor and function as a relatively cheap source of cues to meteorological and tectonic activity, dust and sand storms, even animal life.
 - Solar spectrum in UV and IR.
- 7. Insofar as analytical instruments can be perfected, the humidify of the atmosphere and of soil samples; pH and conductivity of the soil when saturated with added water to furnish data on electrolyte content. The last item could



give some clue as to the importance of volcanic emission and of aqueous erosion in the earlier history of the planet.

8. The microbial culture device might still be justified as an alternative or backup to the microscopic examination, particularly if the communication parameters are altered during the development of the vehicle. Although this approach would command a second priority in terms of the concept, the present paper may still be pertinent and may become even more so for other ventures.

CYTOCHEMICAL STUDIES OF PLANETARY MICROORGANISMS

Explorations in Exchiology

A research proposal to the National Aeronautics and Space Administration submitted by

Joshua Lederberg . .

Professor of Genetics Stanford University Medical School of Medicine Palo Alto, California

April 1, 1961 to March 31, 1964

Total Amount Requested for Three Years

Direct Costs: \$331,000

15% Indirect Costs: 49,650

380,650

Signed for Stanford University:

EXPLORATIONS IN EXOBIOLOGY Introduction

Since March this year, I have been responsible for a preliminary project with NASA support with the aim of defining the reasonable objectives, means and scope of the effort we might contribute to the development of instrumentation for the detection of planetary life. The general approach that we have adopted to this problem is outlined in previous reports and publications; for guidance here we have been greatly helped by the exobiology subcommittee of the Space Science Board, and by consultations with members of the NASA headquarters committees and with the staff at the Jet Propulsion Laboratories. In brief our efforts would be focussed on the detection of microorganisms, and with their further characterization especially with regard to the presence and role of nucleic acids and of proteins. The devices are intended for use on projected landing missions to Mars and Venus in the period from 1964-1967, that is for the interval when we have definite but restrained capabilities for landing automated instruments, and for telemetric retrieval of data, but before we can expect to recover actual samples of planetary materials. However, the experience would also be relevant to general studies on the moon, and to the analysis of return samples from the moon and the planets.

The present application is a proposal for the long term support of these studies in the directions and on a scale that we now feel to be the most appropriate for our participation. It would allow for the close cooperation of two senior investigators (myself as experimental biologist, and an electrical engineer or applied physicist for the instrumentation) and one research assistant or technician in each of our respective fields, together with some essential further assistance as outlined in the budget, appended. There is room and intention in this program for the participation of graduate students, whose academic programs can be accomodated through the degree programs in Biology, Biophysics, Genetics or Electrical Engineering at Stanford as best befits each student's needs. While the present program is sharply focussed on exobiology, it may help serve as a prototype for the fuller utilization of electronics in experimental biology and medicine. My own recent experience on this project has helped me to realize how little of the potential values here are being exploited, partly because of the understandable conservatism of university research workers in biology and medicine to request levels of financial support requisite to the purpose.

It is possible, in fact definitely expected, that some of the fruits of the present program will have useful applications in terrestrial science and medicine. We assume that the NASA (as would other research-granting agencies) would encourage the development of these incidental fruits, especially insofar as they contributed to the success of the central program. For example, a rapid monitor for microorganisms could also serve as an alarm system for the surgical theater on one hand, for biological warfare defense on the other! Conversely, investigations in these fields have laid the groundwork for the intended study of planetary organisms. Of course, major diversions from exobiology would be supported independently.

Proposal

With the research team herein indicated, we propose to continue to study the feasibility of several related approaches, and to outline the appropriate instruments. As far as our intellectual and material resources allow, we will build and test prototype instruments. The shops of the Department of Electrical Engineering are available for the purpose; we will however also have to rely heavily on the cooperation with NASA laboratories for solid hardware.

Four approaches are now in view; however, an important reason for building this team is to allow as to look at others that we have not had time to look into adequately.

- 1. A culture device ("multivator") comprising a variety of nutrient media; microbial growth would be detected by the scattering of incident light into a masked photodetector. This is a simple concept which is ready for prototype construction at a Space Administration Laboratory; we might help to improve on the preliminary designs, and to test out the device on a variety of terrestrial samples.
- 2. The detection of microbial enzymes in soil samples by micromethods.
 We have done no work on this so far; several possibilities are open for the detection of such characteristic enzymes as nucleases and adenosinetriphosphatase.
- 3. The concentration of microbes from soil and their characterization for DNA and protein by semi-micro photometry. This may be plausible as one way to examine the output of flotation methods for concentrating microbes, and would be technically much simpler and therefore available for smaller payloads, but probably less sensitive and specific than 4. New information on the spectro-refractrometry of bacteria is needed and will be part of the work.
- 4. The examination of single particles by microscopic and microspectrophotometric methods after preliminary fractionation of soil. This is the most
 substantial program, as outlined on the attached scheme. Preliminary work has
 been done primarily on the microscopy; this scheme would be the main challenge
 to the engineering research associate.

Some recent progress

Although we have had the preliminary grant for about 6 months, I have only recently decided to make a deliberate long term commitment for personnel, as would be entailed in employing an engineering associate in the laboratory. We are now interviewing several promising candidates — the program has attracted a number of inquiries. We therefore have relatively little to offer on this side; also, even rudimentary equipment has been subject to vexatious delays in delivery. The following points can be made:

- 1. Television display (commercial standards) is entirely adequate for work in biochemical microscopy. We are studying the <u>minimum</u> standards for conservation of bandwidth: probably about 250 lines and 2-3 shades of gray will be reasonably adequate.
- 2. Existing vidicon camera tubes, in connection with readily available light sources, are <u>not</u> sensitive enough for critical UV-microscopy. However, developments now in progress (e.g. the Westinghouse Ebicon) would alter this conclusion.
- 3. The original proposal for UV-microscopy can be substantially improved. UV optics should be used for <u>spectral</u> absorption information; phase optics or transmission optics on stained material for <u>image</u> formation. (We are studying the design problems of fast scanning UV microspectrometry.)
- 4. Microorganisms can be (remarkably) efficiently concentrated from soil by flotation, currently in colloidal silica sols (Ludox). The success of this tactic is optimistic for a program of direct observation of planetary soils, obviating the need for difficult inferences on how to culture them.
- 5. (Most recently) Polyvinyl alcohol may be the ideal material for plastic films intended to hold specimens for microchemical study. The films are optically satisfactory, even to transparency in the UV. They dissolve slightly in water, which is to great advantage since the moistened, tacky films will entrap dust (microbes); the evaporation of added moisture leaves a film in which the particles are firmly embedded. Basic stains, e.g. methylene blue, can even be incorporated in the preformed film at concentrations that will differentially stain bacteria. Thus a bacterial suspension can be sprayed on a methylene-blue/polyvinyl alcohol film, then allowed to dry. Without any further treatment, the stained bacteria are readily discerned (the stain must, fortunately, have a low avidity for the plastic and be readily concentrated by the bacteria). As the seeded films can also be hardened by exposure to formaldehyde, probably even better ethylene oxide,

the system opens the way to easy processing with other water soluble reagents, e.g., enzymes, for cytochemical characterization.

(6. A fortuitous coincidence. Polyvinyl alcohol films stained with iodine have been reported by Hoshino and Yoshida to make useful ultra-violet filters. In a few weeks' work we have been able to improve the performance of these films very markedly (viz. to transmissions of > 50% at 2500 Å, << 1% through most of the UV) to the point where they will be extremely useful for UV microscopy. The best interference filters now evaluable near about the same half bandwidth, and only about 15 per cent transmission.)

Budgetary Comments

The costs of instrumentational research in this area are very high, both in respect to the salaries commanded by engineering specialists, and the purchase of already available equipment where this is available. For example, Zeiss is advertising a prospective (1961-1962) microspectrophotometer designed by Caspersson, many of whose elements would be very pertinent to our needs, but will require substantial adaptation, at \$41,000. Television, recording, and other optical and electronic equipment are likewise very expensive. The budget attached hereto, large as it is, would not be realistic except for the prospects of close cooperation with the Jet Propulsion Laboratories and with other units of the NASA, particularly if a NASA Life Science Laboratory is established in the San Francisco Bay Area. This cooperation should make it possible to use the most expensive hardware more efficiently, and to relieve the present budget of the much greater expenses of ultimate adaptation of prototypes to the engineering requirements of spaceflight.

The item for travel is intended to include travel by the senior personnel, for technical purposes including travel on NASA consultations, which will often be combined with the conduct of project business. Foreign travel, subject to such regulations as NASA may indicate, is also contemplated, primarily to meetings of COSPAR or other international bodies dealing with space science; such travel would be important to keep abreast of the plans for space experiments in other countries, and also to consult with biophysicists in other countries for example, Walker (Edinburgh), Caspersson and Thorell (Stockholm) who can make Important contributions to the Instrument development. The funds should also be available (for cautious and deliberate use) to bring such people to our laboratory where they might be of unusual help in solving specific technical problems.

At the present time, a laboratory area of about 800 Ft² is available for the exobiology research. This space is "on loan" pending the construction of a new laboratory building which will house research in the field of "molecular medicine". It would accelerate the construction of this laboratory, which would be a definite improvement for work in exobiology, and especially in integrating it with other research in the Department, to have a commitment for the pro rated cost of construction and equipment for space allotted to exobiology. The item of \$50,000 is the anticipated cost for about 1000 Ft² fully serviced and furnished, to be available about 1963.

BUDGET - EXPLORATIONS IN EXOBIOLOGY

	Annual rate
Personnel	(aurit edi
Principal investigator - Prof. J. Lederberg (Microbiology) Senior Research Associate (Biophysics; Instrumentation) -	by Stanford
according to qualifications, to Research Assistant - Microbiology, Biochemistry - Biophysics, Electronics	\$16,000 6,000 7,000
Mechanician Secretary (and purchasing agent!)	6,000 5,000 other funds
Graduate Students Equipment	25,000
Laboratory instruments and furniture; Custom fabrication (including design consultation fees) of laboratory prototy	pes
Supplies and technical services	10,000
Chemicals, glassware, medla Small tools, instrument parts Office supplies; photography and drafting	
Communication and travel	4,000
Reference materials	2,000
Technical information, books and journals; publication and reprint charges	
Construction and remodelling	1,000
Basic Annual B	udget = \$82,000

Three Years at 282,000		\$246,000
Non Recurrent Items: Additional Equipment First Year Prorated Construction Costs of New Labs	1963	35,000 50.000
Direct Costs		331,000
Per cent Indirect Costs		

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Report to the National Aeronautics and Space Administration "Cytochemical Studies of Planetary Microorganisms - Explorations in Exobiology" NsG 81-60 (S1)

April 1, 1961 - February 28, 1962

Department of Genetics Stanford University School of Medicine Palo Alto, California

Principal Investigator

Elliott Levinthal Project Director

8

A. Experimental Program

The experimental work during this period falls into four areas:

- 1. Multivator hars Mariner B Experiment
 - a) Low level fluorometry instrumentation
 - b) Biochemical assays and soil chemistry
- 11. Concentration of Bacteria
- III. Fluorescent Staining
- IV. High speed scanning UV microspectrophotometer

A. Experimental Program

1. Multivator - Mars Mariner B Experiment

The next opportunity for the investigation of Mars will be at the February 1965 opposition. Tentative plans might allow for a flight of about six months duration starting November "December 1964. This mission, Mariner B, might release a reentry capsule to deposit an instrument package on the Mars surface in May 1965. Engineering planning for this mission is still in progress and final decisions on its details, or whether it can be flown in this configuration have to be made.

Multivator designates a small automated laboratory being developed in collaboration with the Jet Propulsion Laboratory (George Hobby, cognizant scientist; Jerry Stewart, cognizant engineer), and the Exobiology Group at Stanford. It is designed to retrieve samples of surface dust and inject 100 mg of this material into each of some twenty modules. Each of these comprises a pair of 1 ml reaction chambers together with stored reagent solutions. The simplest programs would be preferred, but some additional process steps may be included through the use of 1) delayed-release of microcapsules of reagents, 2) shaking and sedimentation through centrifuge action, already part of the injection mechanism, and 3) the use of semi-permeable or semi-soluble membranes and filters to partition compartments within the reaction chamber. The reactions can be read photometrically, e.g., for absorption, fluorescence, scattering, polarization, color, or radio scintillation. Other detectors suitable from the standpoint of size, power, complexity.....are also available, e.g., for conductimetry, pH measurements, solid state counters. The signals would be telemetered directly to the earth. An important consideration in the design is the limited telemetry rate, about one bit per second, imposed by the available power: distance ratio.

One of the impelling motives in designing this device is to establish a new interface for laboratory scientists interested in future space research: the individual module in a Multivator rather than the national space program.

The main aim of our present work is the detection and characterization of Martian life. We have to balance what we would like to measure with what we are able to manage within the limitations of the experimental system, taking account of the costs in money, time, and effort of new development work and their offset by other more general uses.

We have concluded that the experimental limitations preclude the direct compositional analysis of a 100 mg soil sample for living forms in any reasonably expected abundance (say 1000 bacteria per sample). Such a sample of terrestrial soil might have a few micrograms of DNA or protein whose detection would be a formidable problem with all the resources of an earthbound laboratory and would still leave knotty questions of certainty of identification. Instruments to deal with such

challenges to microchemistry should be developed for further missions. Hearnwille we have concentrated on <u>functional</u> tests for growth, and for enzyme activity that give higher sensitivity but require riskier guesses as to the functions that should be sought and the conditions in which they will be manifest. In addition, some of the modules will be devoted to some elementary determinations of the physical and chemical properties of the sample.

The Mariner B capsule instrumentation package also calls for measurements of the temperature within the device and such environmental parameters as atmospheric pressure, illumination and UV flux, oxygen CO, and humidity.

The enzymatic tests fall into these categories:

A. Hydrolytic enzymes by fluorometry testing for the catalysis of RX + H₂0 -> RH + XOH. For example, R = phosphate, X = alpha naphthol to constitute a fluorometric assay of phosphatase. This enzyme is given special emphasis in our planning as (1) a ubiquitous occurrence in terrestrial organisms, (2) catalyzing a type reaction of only moderate specificity, (3) indicating a significant role of organic phosphates in the reacting material, (4) readily accessible to ultra-assay.

The basis of the test is the release of XOH which differs from XR in being highly colored or, better, fluorescent. In place of naphthol, fluorescein derivatives are now being studied-they are potentially about 100 times more sensitive in discrimination against background.

- B. Depolymeroses: These assays take advantage of the retention of C¹⁴-labelled polymer by a distysis membrane. Distysele products would furnish counts to a scintillation packet or other counter. The presence of proteases, nucleases, etc., would be good evidence of the role of the corresponding polymer, but at present seem to be better cargets for analytical determination. An analogous method is applicable to other inschable substrates, e.g., phosphocallulous for phosphatase, SOO for sulfur utilization.
- C. Decarboxylases and pas-yielding matabolism. Polyethylene and other polymers form films with interesting permeability properties, e.g., the selective flow of CO₂, CH₄ and other gases to the exclusion of larger, polar solutes. We are studying the application of such membranes to the detection of metabolic C*O₂, C*O, or C*H₄ from C¹⁴ labelled nutrient substrates.
- D. <u>Miscellaneous assays</u>. We are considering, but for reasons of technical difficulty or conceptual bafflement are not overenthusiastic about tests for other important metabolic sequences, e.g., hydrogenase, catalase, photosynthesis, and the utilization of inorganic substrates.

Many enzymes could be assayed by the familiar trick of coupling with DPN Coupling DPNH; in general we would prefer to avoid sending one enzyme to chase another, and it would be risky to rely on the involvement of DPN on Mars. However, these systems deserve much more consideration than we have given them, especially to exploit the fluorometry of DPNH.

The following is a list of experiments that are being studied for possible inclusion in the Multivator:

Soil Chemistry and Physical Measurements

1. A. Dry soil:

conductivity (moisture content)
specific gravity (by radio-attenuation).

B.* No-soil control

2. Soil + water:

conductivity (electrolyte content) turbidity

turbi pH

Reagent for Fe:

color-fluorometry; details under

development

4. Reagent for silicate/carbonate:

color-fluorometry; details under

development

Phosphatase Assays

5. A. Soil + reagent:

pH 8 (fluorometry) (conductivity)

B! Soil + reagent:

pH 5

6. A. Soil -----

(background fluorescence)

B. (phosphatase) reagant

calibration test on known phosphatase

7. A. Soil reagent + phosphatase

(inhibitor in soil?)

Bt Soil Fluorochrome

fluorescence quencher in soil?

B. A. Soil + iodine + reagent

enzyme? sensitive to poison?

B! Soil + reagent + pH sweep

9. A. Soil reagent

pH 8 temperature sweep

B. ---- reagent

pH 8 remperature sweep

10.

Analogous Fluorometry

11. 3'-;5'- nucleotidase

(naphthyl adenylate)

12. deoxynucleotidase

(naphthyl-thymidylate)

13. leucine amino peptidase...leucyl nepthylamide substrate ----> fluorescent alpha-naphthylamine

14. sulfatase

naphthyl sulfate

15. esterase

naphthyl butyrate

B-glucosidase...

naphthyl glucoside

^{*}Unless otherwise specified*, cell B of each module is a reagent blank control (no soil).

Depol	ymerases	Ьу	Dialysis-scint! lometry
21.	DNA		

cl4 or P32 labelled substrates

- RNA 22.
- 23. protein
- 24. synthetic L-polypeptide
- 25. synthetic D-polypeptide
- 26. cellulose
- starch 27.
- 28. mixed biological polymer
- synthetic anomers 29.

(ci4 algae...residue after dialysis).

e.g., poly-L-glucose if available

Growth and Metabolism

nutrient broth 31.

C14 nutrient broth 32.

acetate + C12 bicarbonate 33.

34. amino acids

c14 35. glucose

c¹⁴ acrose (mised sugars from 36. polyformaldehyde)

s35/dialysis 37.

turbidometry of compartment across porous filter. Monitor pH and conductivity

C*0, by selective permeation

CC_o fixation (decarboxy!asa)

utilization of free sulfur

Miscellaneous Systems (for development)

41. ATPast

42. DPN reductase

43. catalase P³² phosphate by desorption or "chromotography"?

? choice of electron acceptor,. Ho? DPNH by fluorometry

0, by gas-permeated, fluorescencequenching

NOTES:

- 2. Feasibility of titrating available OH and Ht is being considered. pH measurement before end after solution of microespaules might serve but will require additional controls.
- 3. Fe₀0_x (limonite) is often cited as the probable basis of Mars' red color, and if this is so should be particularly abundant. Polarographic measurement might be the most direct, if we want to avoid colorimetry (2-wavelength differential photometry) of well known reactions. Fe+++ also quenches fluorescence of many ayes, and reversal by SCN might be diagnostic.
- 5-9. Phosphatase is the first candidate for this intensive study. The same effort could be transposed to any other worked out system.

- 5 A. Conductivity readings will monitor the achievement of the operational program of soil and reagent injection.
- 5 B. Check: acid vs. alkaline phosphatase.

8.

Ø.

- 8 A. This deserves more thought. What would be the most informative inhibitor?
- 8 B. To locate optimum pH of enzyme. By gradual dissolution of microcapsules. Redundant (cf. 5 A vs. 5 B) and tricky but more informative.
 - 9. If one chamber can be heated without prohibitive power load. The temperature at pH sweeps will locate optimum most useful in planning follow-on experiments.
- 21. These assays can also be done fluometrically, by release of adsorbed fluorochrome dyes, but one should anticipate higher blanks. To increase sensitivity polymers can be partially predigested, then exhaustively dialyzed to leave barely impermeable fragments.
- 31. Growth is also indicated by the kinetics of enzyme activity in continuous measurement. When we can learn to measure growth new dimensions are available: nutrition, inhibition, enough material for compositional analysis. Would we dare rely on this now?
- The modules as now designed do not lend themselves to simultaneous turbidimetry and scintillometry. If another counting method is used, these experiments might be compressed. C*O or C*H, would also be measured. If soil iron is Fe⁺⁺⁺ this may be the most plausible oxident. Such uncertainties as to the overall metabolic cycle speak in favor of concentrating on isolated metabolic steps.
- 43. But will a few hacteria add appreciable 0₂ to the background from spontaneous decomposition of H₂0₂?

Our present experimental efforts in connection with the Multivator for this past period have been concentrated in the particular area of fluorometry and especially with the phosphatase assay. These aspects of our work are discussed below. During the next period we hope to define and test many of the other assays to be carried out in the Multivator.

a) Low Level Fluorometry instrumentation

Techniques for very low level fluorimetry have been investigated to some extent. There appear to be two limits to the lowest attainable level of the detection of activity by the use of fluorescent compounds.

The first limitation is instrumental. In the laboratory, the intensity of the light source and the sensitivity of the detector may both be made sufficiently large as not to be the limiting factors. The limit of detection is set by the background fluorescence of the various components of the sample compartment and scattered excitation light which gets through the secondary filter. These effects may be minimized by the proper unulca of geometry and components. This background level appears to be on the order of 10⁻⁸ to 10⁻⁹ of the input light in the best presently obtained configuration.

The second and more important limit of detection is in the substrate-fluor-system itself. Here the residual fluorescence in the substrate and the background introduced by the soil being investigated are two to three orders of magnitude higher than the instrumental background.

For the low level determinations a Bausch and Lomb "Spectronic 505" spectrophotometer has been converted for dual beam fluorescent measurements. The conversion is such that the machine may simply and quickly be returned to its normal spectrophotometric function. (See Figure 1).

Light sources for laboratory and spacecraft applications have been and are being investigated. Brightness, power input, efficiency, spectral distribution, and physical size are the criteria of interest. Investigations of gas discharge, tungsten and fluorescent lamps have been made.

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b) Biochemical Assays and Soil Chemistry

Two methods of assaying for phosphatese activity have been investigated; colorimetric, using p-nitrophenolphosphate as the substrate, and fluorometric, using a-napthol phosphate as the substrate.

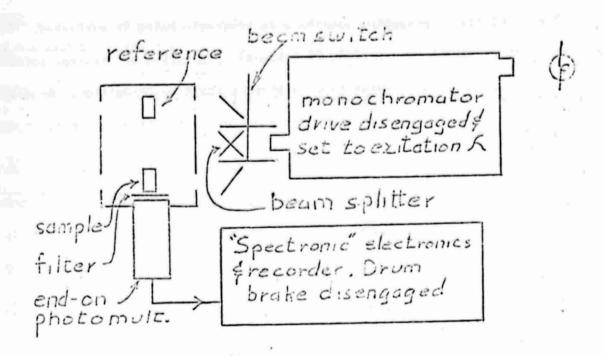
The colorimetric assay which can detect about 6×10^{15} molecules per milliliter* p-nitrophenol as a lower limit is not sufficiently sensitive so as to measure bacterial populations of the order of 10^2 . As a consequence, the colorimetric assay is not presently being pursued.

The fluorometric assay has been studied with respect to the following factors; soil fluorescence, the concentration of a-napthol phosphate required for maximal activity, the fate of a-napthol, and the stability of a-napthol phosphate.

To date, two samples of soil have been investigated with respect to their background fluorescence. One, an arable soil, fluoresced at a level equivalent to 8.4 x 10 cml alpha-napthol when present as a 10 per cent suspension. This represents 6 per cent of the total fluorescence observed when the same soil is incubated with a-napthol phosphate for one hour. On the other hand, the fluorescence of a death valley sand was less than 1.8 x 10 cml a-napthol equivalents (when present at the same concentration as the arable soil). Further studies are in progress designed to ascertain the inherent background fluorescence of various soils and to determine whether any simple procedure may distinguish between fluorescence due to organic and that due to inorganic materials.

A series of studies have been conducted to determine the fate of a-napthol in the soil. When mixed with soil, a-napthol rapidly disappears; the time course of disappearance showing two distinct phases—an initially rapid rate followed by a slower rate of disappearance. The rate of disappearance is proportional to the concentration of a-napthol from 0 - 10-5M (no greater concentration was tested). The amount of disappearance also depends upon the amount of soil. Attempts to extract

Mortcules per milliliter hereinafter abbreviated cml (i.e. chemical units per ml.).



Spectronic 505 as arrunged for fluorometry

Originals force in Or ends oliging a-napthol from soil after it had disappeared has not proven too successful. The following solvents have not extracted the bulk of the a-napthol; methanol, ethanol, isopropanol, acid-ethanol, and alkaline-ethanol. Although it is possible that the disappearance of a-napthol is a result of its degradation by biological (or even non-biological) agents present in the soil, this may not be entirely true since ethanol extraction does succeed in extracting a small amount of a-napthol-like material, and the kinetics of a-napthol disappearance seem contrary to what one would expect if a biological degradation occurred.

The stability of a-napthol phosphate is presently being investigated. To date the following observations have been made. In solution, a-napthol phosphate is reasonably stable for short time periods (1-2 hours) in alkaline and neutral solutions when kept at room temperature and exposed to visible light. On the other hand, irradiation with 336 mµ light results in a relatively rapid decomposition. A-napthol phosphate decomposes slowly to a-napthol when kept at either 9°C. or -10°C. for extended time periods. Currently, the stability of a-napthol phosphate when stored in vacuo is being investigated.

At the present time, the following conditions are employed in the phosphatase soil containing approximately a count of 10° bacteria per gram. From 0 to 1 gram of soil is incubated with 2 ml. $5 \times 10^{-9} M$ a-mapthol $(3 \times 10^{18} \text{ cml})$ phosphate for 1 hour at room temperature. The soil a-mapthol phosphate suspension is gently agitated during incubation. Following centrifugation, an appropriate aliquot of the supernatant is brought to pH 10 with NaOH and the fluorescence resulting from excitation at 336 Mµ is measured at 460 Mµ. In a typical experiment, 200 mg. of soil when incubated with 2 ml. a-napthol phosphate yielded 1.5 x 10½ cml of a-napthol. The background fluorescence of this soil (200 mg. soil incubated with 2 ml. distilled water) was equivalent to 8.4 x 10^{14} cml a-napthol. Thus the background was equivalent to about 6 per cent of the total fluorescence observed, while the total fluorescence represented about a 0.5 per cent conversion of substrate to a-napthol. The instrument background limited the detectibility to 6 x 10^{12} cml equivalents of a-napthol for a S/N=1. It should be pointed out that these data have not been corrected for a-napthol disappearance.

It can be calculated from the above that a count of 1 bacterium per ml. would catalyze the appearance of 1.5 x 10^8 cml of a-napthol from a-napthol phsophate in 1 hour. Assuming that in a direct count of soil approximately 10 per cent of the count are viable cells, then the observed rate of phosphatase activity should approximate 1.5 x 10^9 cml per hour per viable cell. The intensity of fluorescein fluorescence is approximately an order of magnitude greater than a-napthol (giving an instrumental sensitivity of 6 x 10^{11} cml) and, since its excitation frequency is in the visible, it should give less difficulty with soil background. It is possible, therefore, that a fluorescent phosphatase assay could have a \$/N=1 for a viable bacterial population of 4×10^2 bacteria per ml. per hour using the existing instrumentation and a suitable fluorescein derivative and assuming no difficulties with soil background.

A summary of the above data is as follows:

nstrument Sensitivity cml for S/N=1)	Background (cml)
6 x 1012 6 x 1012 6 x 1011	≤10 ¹² -10 ¹⁴ ≤10 ¹¹
	cml for S/N=1) 6 x 10 ¹⁵ 6 x 10 ¹² 6 x 10 ¹¹ 10 ⁹ molecules/hour/viab

The above instrumental sensitivities are those we have achieved with a minimal effort. Further work is being done on the instrumentation to reach sensitivities closer to those theoretically possible. It should be pointed out that it is not justified to extend the sensitivity beyond the limitations imposed by background noise.

II. Concentration of Bacteria

To date, two methods have been employed; concentration of cells based upon centrifugation in media possessing densities greater than water but less than those of bacterial cells (density separation), foam flotation.

Density separation has so far proven inadequate using the following agents to increase the density of the suspending medium: CsCl, sucrose, glycerol, and various modifications of Ludox (LS, HS, and AM). In the case of Ludox, concentration could be affected when soil-free suspensions of bacteria were employed. Thus, a suspension of bacteria occupying a height of 2.5 cm. could be concentrated by centrifugation in Ludox HS for thirty minutes at 500 x g to yield a zone of concentration about 1-2 mm. in height. When soil suspensions containing about 10 bacteria per gram were suspended in Ludox and treated in an identical manner the results were erratic. In many experiments a substantial number of bacteria were floated, but many remained in the sediment. In other experiments, very few floated. These effects may be due to the adhesion of microorganisms to soil particles, and some improvement of separation follows after preliminary mixing of the soil in water. However a one-step procedure would be preferable. Varying the Ludox concentration, the ratio of soil to Ludox, pH, and the time and force of centrifugation did not improve the separation.

The second method, foam flotation, appears fruitful. As presently operated, a suspension of soil is placed in a gas washing bottle containing a coarse porosity filter and Tween 80 is added as the collector. Foaming is induced by the positive application of air. Although the concentrate does not exhibit a significant concentration of cells relative to either the feed or the tailings, it does contain less soil than either the feed or tailings. At the present time, efforts are being directed along lines designed to increase the concentration of cells during flotation by varying the nature of the collector, the pH of the feed, the ionic strength and type of ions in the feed, and the time and rate of foaming.

At the present time further efforts along the lines of developing the density separation and foam flotation techniques have been diminished owing to the pressure of the Mariner B Multivator program and the expectation that these techniques will not be required on that program.

III. Fluorescent Staining

An attempt is being made to devise a simple and rapid fluorescent method for staining bacteria directly in the soil. To date, the only fluorochrome employed has been Acriding Orange. Under the presently defined conditions (Acridine orange, 10⁻⁴M and NaCl, 10⁻³M), soil-free bacterial suspensions stain rapidly merely by mixing a loopful of the bacterial suspension with a loopful of Acridine orange-NaCl on a slide and observing fluorescence with a dark-field UV microscope. An anomaly appears under these conditions: some cells fluoresce green while others on the same slide fluoresce red. When bacteria taken from a Winogradsky column are stained under identical conditions, they all fluoresce green. It should be pointed out that the cell types are not the same, and this may play a role. However, at the present time no reasonable explanation for this behavior is available. Studies on the utility of fluorescent staining are being continued along the following lines; clarification of the nature of the dual staining, further optimizing the conditions of staining, and eventually investigating other fluorochromes.

IV. High Speed Scanning UV Microspectrophotometer

The central element is a flexible camera control to drive the image tube at specified rates to optimize the trade-off between speed and sensitivity as well as to investigate different raster putterns. The digitized camera control for variable rate and raster pattern has been constructed and is now in its final stages of test and evaluation. Operational amplifiers with high-current output stages were required to drive the vidicon deflection coil. The use of low-inductance deflection coils and direct-coupled current-feedback amplifiers permits the beam to traverse the raster area in either direction in about 5 µs, and, at the other end of the scale, to sweep arbitrarily slowly. Some difficulty remains in the form of inter-axis coupling. The problem will be eliminated in the future by use of a larger camera box with space for amplifiers, etc., thus eliminating low-level signals in long cables. The difficulties caused by this coupling will not prevent useful application of the present configuration. Fail-safe deflection-dependent unblanking is incorporated.

Counters, logic, and digital-to-analog converters provide a choice of four raster formats, (see Figures 2,3,4,5), three of which alternate lines between the upper and lower raster halves. The number of lines may be any power of 2 from 1 to 12: i.e., between 2 and 4,096. The fraction of the raster area which is scanned may be any power of 2 from 0 to -11. The horizontal sweep may be generated either by the integrate-and-trigger method, which permits rates from standard TV speed down to arbitrarily slow, or by means of a delay line.

The rasters are attained by counting the horizontal pulses (at line rate), and driving a binary digital-to-analog converter with the contents of the counter. The converter drives the vertical-deflection operational amplifier. The number of lines is determined by where along the counter chain the horizontal pulses are fed in; i.e., of all 12 counter stages are used, 4,096 lines result. If the input is applied to the fourth stage from the end, 16 lines result, etc.

The fraction of the raster area which is scanned is determined by where the counter reset signal comes from. If the output of the last stage resets all the counter stages, full scale output is attained by the converter, and the whole raster is scanned. If the reset signal comes from the next-to-last stage, the count stops half-way, and the converter and deflection attain only half of full scale.

The raster format is determined by the logic which is connected between each stage of the counter and the corresponding bit of the digital-to-analog converter. A conventional staircase raster (Figure 2) results from simply connecting each counter stage to the corresponding converter switch.

To get the oddly displaced staircase (Figure 3), in which each odd count is displaced by half of full scale, all bits of the converter, except the large bit, are driven directly from the counter, as before; but the large bit of the converter is driven by the small bit of the counter. Hence, the converter's output jumps up or down by half of full scale every line. Note that there are only half as many lines as before. This is because, at the converter, it is impossible to have the large and small bits different: half of the count combinations are removed, and the converter output cycles twice for each counter cycle.

The symmetrically converging raster of Figure 4 is attained by driving the large bit of the analog-to-digital converter from the small bit of the counter, and driving all other converter bits with the non-identity function (the "exclusive or") of the corresponding bit of the counter and the small bit of the counter. Again, there are half as many lines as counts but for a different reason: the non-identity

function of the small bit with the small bit is always false, so the small bit never changes in the converter and half the count combinations are removed. This does not result in each vertical deflection position lasting for two line times, as one might expect. Since the small counter bit may change all except itself, a change occurs for each line.

The symmetrically diverging raster of Figure 5 results from driving the large bit of the converter from the small bit of the counter, and driving all other converter bits with the negation of the exclusive or (identity) of the corresponding counter bit and the small counter bit. Again the number of vertical deflection positions is half the number of counts, for the same reason as in the preceding case.

With delay-line sweep generation, video may be superposed upon the synch in the delay line, thus giving one-line synchronized video delay. Line times from 63 μs to 250 μs are attainable with the present delay line. Consideration is being given to the use of a more flexible delay device for our laboratory investigations, such as a video storage tube.

Video log-difference circuits for ratioing the sample signal and reference signals have been completed. Tests of the electronics with a microscope input are now being initiated.

The optical train for the high speed scanning ultraviolet microspectrophotometer has been assembled and still photographs have been made through the system to determine optical parameters. The Zeiss ultraviolet optics appear to be quite adequate.

B. Personnel and Organization

Mr. L. Lee Hundley and Dr. Lawrence Hochstein have joined the laboratory. Dr. Hochstein is a microbiologist and is responsible for that phase of the activities. Three biology honors students are working in his laboratory and we have employed a research assistant to replace Mr. Elliot Packer who has just left to complete his graduate studies.

In addition, we have employed an electronics technician and a machinist who has organized a small model shop. We are seeking to expand the staff by hiring an analytical chemist. This will allow a concerted effort on the detail design of the Multivator assays.

In summary the organization of the project is now as follows:

Professor J. Lederberg - Principal Investigator and Head, Genetics Department

Dr. E.C. Levinthal - Project Director

Lee Hundley - Electrical Engineer

L. Hochstein, Ph.D. - Microbiologist

Harrison Horn - Electrical Engineer

-----.Ph.D. - Chemist

3 additional supporting personnel full time

3 part-time assistants (Biology majors)

FIGURE 2

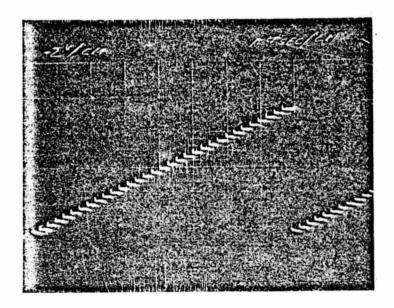
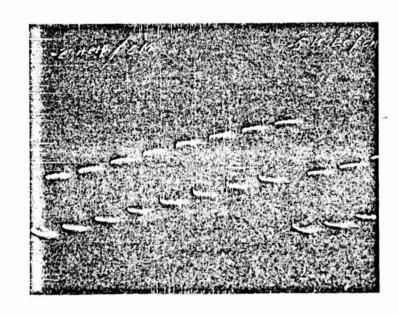


FIGURE 3



OK POOK WALITY

FIGURE 4

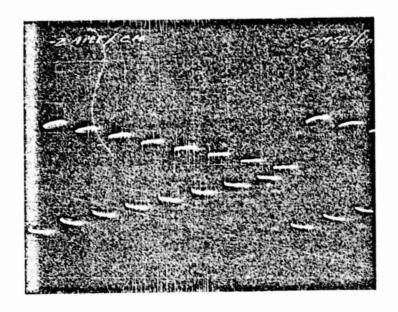
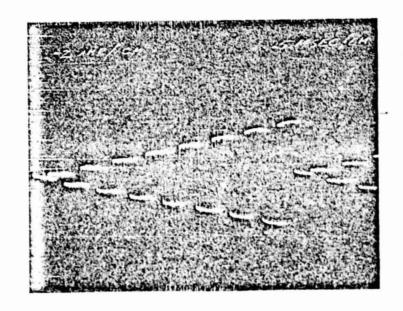


FIGURE 5



MEDICAL CENTER PALO ALTO, CALIFORNIA

DEPARTMENT OF GENETICS
School of Medicine

May 1, 1961

DAvenport 1-1100 Cables STANMED

The Honorabie Lyndon B. Johnson Vice President of the United States Washington, D.C.

Dear Mr. Vice President:

Mr. Kennedy's recent remarks were of very great interest to every
American concerned with the national space program, and, with your indulgence,
may perhaps at least serve as an occasion for this letter.

There is one mission within the reach of our present vehicles that, with some luck, could help us regain the initiative. This is an expedition to Mars with advanced instrumentation having the aim of detecting the presence or absence of life.

We have already missed the boat twice. With adequate funding and drive, we might have had at least a fly-by to Mars in 1960 and again in 1962. These missions have withered away and it is probably too late to revive them. The next opportunity for Mars is in 1964, but it will take energetic planning, and funding now, for us to take good advantage of it. I should point out that Mariner B, a very clever and well contrived Mars probe, is now being planned for 1964. The fly-by vehicle will drop a small capsule to the Mars surface and also serve as a radio relay from it to the Earth. But this is a marginal effort. If two or three vehicles could be fired within a brief interval, they would mutually reinforce each other against the risks of a vehicle failure, and in communications. With a single vehicle, communication from the capsule would end in an hour or so as the fly-by goes off. A second relay or orbiter could greatly expand this capability and also allow for a heavier instrument load in the capsule.

Will the Russians beat us to it? Their sturdier vehicles make it hard to compete with them in brute force, but I do not think they begin to match us In instrumentation and communication, and these missions would put great emphasis on these skills. This is especially true in biochemical and basic biological studies where their work is really surprisingly and basic biological studies where their work is really surprisingly backward by comparison with their standing in the physical sciences and in rocket engineering. In any case, they are limited, just as we are, to specific firing intervals in 1962 and 1964. So they have just one chance next year to overshadow our effort. In any case, there would be very great scientific values to an expanded mission — by general admission, far more than might values to various man-in-space proposals, where the Russians have the lead now anyhow.

COPY

There are many other useful and striking things we can do with our space program under vigorous leadership. Unfortunately, there have been such great pressures to develop the vehicles, that we have not been spending the proportionate effort needed to make best scientific and technological use of the ones we have. It is incredible to me that the contract solicitation for industrial proposals to develop a microscope for planetary studies, a rather modest investment, should have been delayed for months towing to a shortage of research funds at the Jet Propulsion Laboratories. Wing to a shortage of research funds at the Jet Propulsion Laboratories. I hope the sense of urgent purpose indicated in Mr. Kennedy's remarks and by your own direction of the Space Council will lead to the proper remedies.

Yours sincerely,

Joshua Lederberg Professor of Genetics

Enc: Clipping from N.Y. Times, April 22, 1961.

President orders top-level review to determine an area in which the U.S. can lead -- Johnson to head study.

"We have to consider whether there is any project now, regardless of the cost, which offers us hopes of being pioneers in a project!

Pres. Kennedy.

ORIGINAL PAGE IS OF POOR QUALITY

INSTRUMENTATION RESEARCH -

STANFORD UNIVERSITY MEDICAL CENTER PALO ALTO, CALIFORNIA

DEPARTMENT OF GENETICS

DAvenport 1-1200 Cables STANMED

May 12, 1961

Dr. Homer E. Newell, Jr.
Deputy Director, Office of
Space Flight Programs
National Aeronautics and Space Administration
520 H Street, N.W.
Washington 25, D.C.

Dear Dr. Newall:

In response to your letter of April 7, I am enclosing 10 copies of a preliminary proposal for the Mariner B Capsule - the "Multivator". This should be thought of as complementing two other approaches to the analysis of suspended particulate material in the Mars atmosphere that are being presented by JPL - the abbreviated microscope and the gas chromatograph. These devices could all hook on to the same mechanism for collection of the dust.

It is obvious that Mariner B is already a highly stressed mission, and it may become much more so as time goes on. I would urge the relevent committees to consider the advantages and technical possibilities of expanding Mariner B to an expedition of two or three space crafts. If these arrive at intervals of a few days the communication capacity of the mission will be greatly enhanced, apart from the assurance of simple backup. I also have indicated this view in the enclosed letter to Vice President Johnson, as Chairman of the National Aeronautics and Space Council.

The political situation here and internationally, suggests the possibility of sudden directives to expand the program on short notice. I urge that we take great care to have thought out potential missions and experiments and supported their development at least to the limits of Imaginable vehicle possibilities, whether or not these are programmed within current plans and budgets. If not, we are bound to be caught with our pants down, and to fail in the best service we can do for our country's goals.

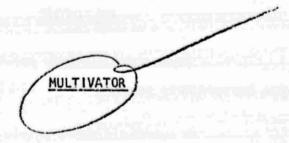
Some specific elaborations that are thought of for the Mariner series include:

- (a) Corroborative altitude measurements for the capsule
- (b) Radar analysis of the surface

- (c) More ample photographic and storage capacity. This could allow for more sophisticated selection of scenes for telemetry, and fuller exploitation of microscopy and other optical methods.
- (d) Wider reconnaissance by the capsule needing longer communication time as well as means of locomotion.

Yours sincerely,

Joshua Lederberg Professor of Genetics



Proposal for Mariner B Experiment - Capsule

May 10, 1961

Submitted by

Joshua Lederberg, Professor of Genetics Stanford University School of Medicine

In cooperation with George Hobby and Jerry Stewart, Jet Propulsion Laboratory

The multivator is a miniature, multiple purpose "laboratory" in which a series of simple measurements can be made on samples of atmospheric dust. A variety of measurements are proposed, and others are to be considered. They have the common feature of testing a small sample of dust with a fluid reagent, and of giving a read-out by simple optical or electrometric measurement. In addition to a determination of solution or suspension properties (turbidity, pH. conductivity) the estimation of phosphatase and other enzymes for which ultramicro-tests are available would be of great help in estimating the possible existence of life on Mars.

MULTIVATOR

The multivator is a miniature, multiple purpose "laboratory" in which a series of simple measurements can be made on samples of atmospheric dust. A variety of measurements are proposed, and others are to be considered. They have the common feature of testing a small sample of dust with a fluid reagent, and of giving a read-out by a simple optical or electrometric measurement. The device was originally conceived to attempt to cultivate Martian microorganisms in defined culture media, but brief communication times make it unlikely that changes based on growth could be observed.

The effectiveness of the multivator depends on 1) the acquisition of samples of surface material from atmospheric dust or from the ground, and 2) the choice of feasible but informative and sensitive tests to conduct on these samples. Part one will be the special responsibility of the development group at JPL. Part two will be the preoccupation of the Exobiology Laboratory at Stanford University. Our continuing studies are based on the assumption that several samples of about one milligram of surface material will be available ("Clean" terrestrial air contains and one milligram dust per 10M3: a 100 km column of 1 cm cross section). Plainly this assumption would most readily be satisfied with access to the ground environment; however, Mars' atmosphere may be dustier than the Earth's. Of the biologically oriented experiments proposed hereinbelow, the determination of phosphatase with the use of substrates that release a fluorescent chromogen appears to be the most promising gamble for the detection of evidence of life.

We propose a device containing 24 test chambers (two circular plates with 12 each) containing about one ml of fluid each and into which the dust sample can be introduced. The plate will be rotated on its axle to allow each chamber in turn to intercept a light beam for photometric measurements. Other chambers will have built-in electrodes for measurements of electric conductivity or potential. The JFL will assume responsibility for and has begun preliminary work on the problems of mechanical design of the multivator and the collection of the dust. The Exobiology laboratory at Stanford will conduct the calibration of suitable test reactions and attempt to develop more sensitive and reliable assays.

The following measurements are now proposed or under review to be

conducted on aqueous suspensions of the dust samples. Refined variants are also indicated. In some cases, the same chamber can be used for several parallel measurements.

- A. Solution Properties
 - 1. Turbidity for rough calibration of further experiments
 - 2. pH
 - 3. Conductivity

- polarographic measurement (help to identify any electrolytes).

- B. Enzyme Tests
 - Phosphatase colorimetric test by splitting of p-nitrophenyl phosphate to release nitrophenol
 - fluorimetric test by splitting of fluorescent phosphates
 - Deoxyribonuclease conductimetric test by release of dialyzable nucleotides from DNA
 - colorimetric test by splitting of p-nitrophenyl-thymidine-phosphate
 - 3. Ribonuclease tests analogous to β2 with RNA substrates
 - 4. Other esterases (suifatase, acylase) and glycosidases by methods analogous to 8/
- c.
- Electron transfer enzymes
- 2. Substrates for electron transfer enzymes
- M'crobial growth on defined media by changes in turbidity, conductivity or pH of the blochemical tests.

Phosphatases (\$\frac{1}{2}\$) are among the most ubiquitous enzymes - 1 know of no tissue or organism that has failed to show them - and they can be readily demonstrated in small samples of soil, dust, sediment from tap water, sea water (about 3 x 10⁻⁹ moles of nitrophenol released per milligram soil per hour). This

is just within comfortable detectivity in a laboratory spectrophotometer, and might also be achieved by differential colorimetry in a compact device. For example, a dual light source might consist of two pin lamps with differential filters, and driven by AC modulated in opposite phase. Differential absorption of one color would be recognized as an AC output from the detector.

This list is oriented to the detection of clues to life. In addition, the measures of part A, together with other microscopic and video reconnaissance studies will be invaluable preparation for the more ambitious efforts of the Voyager series.

We are studying the extension of these microtests for phosphatase and other enzymes as well as possible artifacts and improvements. The presence of phosphatase activity in soil would be presumptive evidence of life related to the utilization of phosphate and can establish the limits of its prevalence.

The sensitivity of this test can be augmented by the use of <u>fluorescent</u> substrates. Dr. Rotman (new working in my laboratory) has shown that the detectivity with these substrates is several orders of magnitude higher than with nitrophenyl compounds and he can readily demonstrate the enzyme level of single bacteria, perhaps even single enzyme molecules.

Equally important and widespread enzymes are the <u>nucleases</u>. We are studying ultramicro methods for these. One possibility is to contain the reaction mixture in a dialysis sac within the multivator chamber, with a film of distilled water in contact with probe electrodes. The release of low molecular weight, dialyzable nucleotides would be signalled by an increased electrolytic conductivity between the electrodes. Whether this can be made sensitive enough depends partly on the electrolyte content of the soil itself.

The instrumental aspects of this proposal can be discussed by JPL. As a preliminary estimate, the design would cost about 5 lbs (mostly the dust collection which might be integrated into the structure of the capsule) and \$\frac{1}{2}\$ watts power. The information output would be of the order of 1-10 bits per second. We are considering further ways of minimizing the weight of the package. The reagents themselves would, of course, weigh only an ounce altogether; and the signal outputs are as simple as from any transducer.

It would, of course, be foolish to delay too long the final decision on

test reactions and the ways these can be incorporated into the multivator. However, the studies on ultramicro assay methods can proceed in parallel with the mechanical and electronic design of the multivator since the basic output from the chamber will be the same regardless of the type of reagent used for the individual test and of the choice between a photometric or more direct electrometric estimation.

The work at Stanford is already adequately funded as an aspect of our current investigations and we are adding additional staff during the next two or three months to help to accelerate them. Additional support may well be required for the instrument development work at JPL. It should be possible to construct at least an elementary version of the multivator with tests and controls for, say, items A, 1, 2, 3 and B1, within the indicated schedule.

February 16, 1962

Dr. Thomas L. E. Smull
Director of Grants and Research Contracts
Office of Space Sciences
Hational Aeronautics & Space Administration
1520 H.Street N.W.
Washington 25, D.C.

Dear Dr. Smull:

At the request of Dr. Joshua Luderberg, I am pleased to forward herewith am application for a grant to construct and equip a Biomedical Instrumentation Laboratory in the School of Medicine. The estimated cost of the facility is \$600,000, and this is the amount requested from the National Aeronautics and Space Administration.

As explained in the body of the application, a new building to be constructed for the School of Medicine is scheduled for completion in 1964. It is highly desirable that the Biomedical Instrumentation Laboratory be included in this building, not only because proximity of the Laboratory to other Medical School activities would enhance the exoblology program, but also because incorporation of the additional facilities in a larger building could well be somewhat less expensive than separate construction of lower quality would be.

Intensive work on plans for the building is now underway. Final cost estimates and architectural plans relative to this proposal are not yet available. As soon as they are, we will send them to you. In the meantime, we will be pleased to furnish whatever additional information would be pertinent to your tavier.

I have reviewed this application as a representative of the University administration, and it has my approval.

Sincerely yours,

ORIGINAL PAGE IS Dean, Graduate Division

bcc: Dr. J. Lederberg OF POOR QUALITY

Dr. E. C. Levinthal Hr. L. E. Cook

Controller

Proposal to National Aeronautics and Space Administration

BIOMEDICAL INSTRUMENTATION LABORATORY

J. Lederberg and E.C. Levirthal

Department of Genetics

Stanford University Medical School

- A. Functions of the Laboratory
 - 1. Expand our present exobiology program
 - Facility for applications of NASA supported effort to other medical school programs.
 - 3. Facility to undertake new projects in space biology
- B. Scope of the laboratory
 - Construction 12,000 square feet (gross) of new construction within Stanford Medical Center
 - 2. Basic equipment, shop and laboratories

\$500,000 100,000

Total facility costs

\$600,000

The facility is intended to support an annual basic rate of research expenditure of \$500,000 among its various programs. These programs are not part of the present application. Our current NASA activities are supported at the rate of \$126,000 per annum (including indirect costs).

Stanford University
December 1961

Requirements for Present Action

A new building in the Stanford Medical Center is now in the final stages of planning, preliminary to architectural drawings. It is planned for completion in 1963-64. The Genetics Department has been allocated new quarters in this new building and will vacate its present ones. The only presently foreseeable way in which we could strengthen our current exobiology program and remain physically a part of the Stanford Medical Center is to incorporate the exobiology laboratory in current planning for the new building. The value of this physical connection cannot be overestimated. It makes possible the indispensable interactions of the excbiology program with the other activities of the Genetics Department and the medical research program of the entire school. These connections are essential for the intellectual and technical support of our own program and for the most efficient applications of our findings to general medical problems.

The estimated cost of providing this additional space in the new building is \$500,000. In addition, \$100,000 will be required for general shop and laboratory equipment. While this building cost represents a higher quality of construction and may be more expensive than might be possible elsewhere on the campus, the increased cost is more than justified by the need for this intercommunication.

General Value of Increased Size

All the functions of the laboratory will benefit from an enlargement of the present program. To be effective it is desirable that an instrumentation research group of this kind contain a broad spectrum of engineering skills. This is important in two dimensions. In the first place, it is important to be able to draw on advanced talents in many different engineering fields, several of which are often involved in one problem of bio-medical instrumentation. In the second place, it is often very desirable to be able to draw on different levels of engineering talent. While it may be an exciting challenge for a Senior Engineer to work on an instrumentation problem through the development and initial construction phase, It would probably be considerably less attractive to such personnel if they felt that they would have to continue for a long period of time spending all their efforts on the operation, use and maintenance of the equipment. An engineering activity should be able to draw on different levels of engineering talent appropriate to all phases of the problem. Many problems, while important, do not justify such a large effort by themselves. In addition, the organization and operation of an instrument development group is foreign to the interests and would be considered a distraction from their other research activities by many faculty members who might otherwise be able to make a significant contribution to NASA's program.

2

1. Expansion of present exchiology program

By completion of the present NASA project we believe that we will have laid a sound basis for increasing our activity in exobiology to about twice its present size, requiring approximately 6,000 square feet of shop, laboratory and office space. Increased activity would be justified because of the need to initiate or continue activities in several of the following areas:

- A. Separation of organisms

 Continue work on flotation

 Foam flotation

 Electrophoresis, and in combination with above

 Flow gating methods
- B. Particle analysis -- microscopic methods

 UV-visible-IR spectrometry: absorption analysis
 Fast scanning methods -- vidicon, etc.

 Microfluorimetry
 Electron-beam scanning of sample: emission analysis

 X-ray emission
 gas emission
 mass spectrometry; ionization detectors

 field emission effects
- C. Semi-micro sample analysis
 Fluorimetry for enzyme assay
 Optical activity and rotary dispersion
 DNAse by viscometry
- D. Search for exotic terrestrial organisms (non-DNA life)
 Selection with nuclein antagonists; P₃₂-incorporation
- E. Application of analytical techniques to Fossils Meteorites Micrometeorites
- F. Establishing laboratory readiness for return samples from moon and planets.
- G. TRAINING of graduate students and fellows in emobiology instrumentation

11. Applications of MASA supported effort to other medical school programs.

We propose to set up a laboratory with physical facilities adequate to support collaborative research instrumentation programs with other departments. In many cases such projects might be supported by individual grants from other agencies such as NiH and NSF which are also concerned with instrumentation, and by cooperation with local industries. The most immediate benefit to our present NASA supported program in exobiology would be those pointed out above, associated with a broadening of our engineering strength. A second benefit would be to insure that the technological skills developed in our exobiology laboratory had a fruitful impact on other activities at the medical center. This possibility can best be illustrated by a specific example.

As part of our exobiology program we are concerned with the problem of acquiring an image of a scene with minimum light level and extracting the maximum information from the video signal with the minimum redundancy. This involves us in the technology of imaging devices such as image intensifier orthicons, video information storage, and signal enhancement techniques. It is easy to see the relationship of this to the problems of fluoroscopy in the Radiology Department. In fact, several discussions have been had between members of the exobiology laboratory and Dr. Herbert Abrams of the Radiology Department, who is already actively engaged in clinical applications of what from an engineering standpoint are already well-developed imaging techniques. A possible research program using more advanced technology is under active, though preliminary, considerations. (An almost identical need exists for processing image information to improve the resolution capability of carth-based telescopes, and we have made preliminary arrangements with colleagues at the University of California to pursue this opportunity to exploit the oppositions of Mars in 1963 and 1965.) Without more laboratory space, our group could not add such projects to its present responsibilities regardless of support for operating costs.

Other examples of such collaborative efforts which are in early stages of discussion involve Dr. Frank Morrell of the Neurology Department, Dr. Halsted Holman of the Department of Medicine, and Dr. Walden Bellville of the Department of Anasthesia

III. New Projects in Space Biology

Several of our colleagues in the medical school have already established research programs under NASA support. There are many others who could make important contributions to meeting NASA's need for basic scientific information and the biological and medical support of space flight. It is fairly characteristic however, that such programs require an insight into the challenges of space biology and access to technical, instrumentational support which are beyond the needs of many otherwise well-qualified research workers. The establishment of the medical instrumentation laboratory with its close functional connection with NASA programs should help materially in providing a channel for the establishment of many new research interests in fields other than exobiology which are highly partiment to the national space program.

MEMORANDUM OF UNDERSTANDING
BETWEEN
NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
AND
LELAND STANFORD JUNIOR UNIVERSITY

CONCERNING RESEARCH FACILITIES GRANT NsG(F) 2-62

It is the policy of the National Aeronautics and Space Administration to support research in space related science and technology at non-profit scientific and educational institutions. Where additional research facilities are urgently needed to conduct such research and the institution involved has demonstrated its intent to seek ways in which the benefits of such research can be applied to the social, business and economic structure of the United States, NASA may supplement research support with funds necessary for the construction of such facilities. The National Aeronautics and Space Administration is particularly desirous that the environment in which space research is conducted will be characterized by a multidisciplinary effort which draws upon creative minds from various branches of the sciences, technology, commerce and the arts.

Stanford University has conceived and implemented a program of research in biomedical instrumentation and exobiology with considerable financial support from NASA. It is expected that the Stanford research efforts in these fields will be quadrupled as a result of space being made available in these facilities. The research results and instrumentation developed are expected to make a major contribution to the nation's space effort as well as finding many practical applications in the medical field. The physical limitations of laboratory research facilities at Stanford are now blocking the expansion of this program in a manner detrimental to the most rapid advancement of the space effort.

Stanford has requested about \$600 thousand from NASA for the support of construction of additional research facilities in accordance with its proposal SC 3363-F and subsequent letters providing additional information. It is contemplated that these new Medical Instrumentation and Exobiology Laboratories will consist of approximately 14,000 square feet of gross floor space and the necessary fixed equipment thereto. These laboratories will become an integral portion of the new Clinical Sciences Research Building connected with the Medical Center. The balance of the funds necessary for the construction of the total 142,000 gross square feet of space to be contained in Clinical Sciences Research Building will be acquired elsewhere by Stanford.

This building will be located on the main campus on land owned by the University, and will become an integral part of the Stanford

ORIGINAL PAGE IS OF POOR QUALITY Medical Center. The convenient location of these and other campus facilities will widen areas of cooperation and contribute to increasing cross-fertilization of ideas and research, thereby enhancing the research potential of the new facilities.

During 1961, expansion of research activities at Stanford was made possible by the increase in the number and size of grants from cutside sources. The University expects a continuing expansion of such activities and that the proposed new facilities will accomposed and be increasingly utilized by both governmental and non-governmental sponsored research in space related science and technology in the ten year period following completion of the facilities.

The proposed new facilities are in accordance with the Stanford long range development program which will eventually enable a substantial increase in the number of graduate students and a consequent increase in the research potential of the University. Ownership of the new facilities by Stanford, instead of by the Government, will assure that control is in the organization which is finally responsible for implementing the long range expansion plans and will eliminate an uncertainty which may be detrimental to the University's fund raising program. Additionally, it is expected that ownership of the facilities will contribute to the execution of the development program and the consequent increase in the University's potential for conducting research in space related science and technology.

Grant No. NsG(F) 2-62 by the National Aeronautics and Space Administration is made for the construction of new biomedical instrumentation research laboratory facilities. Pursuant to the NASA Appropriation Authorization Act of 1961 (Public Law 87-98) the Administrator has determined that the national program of aeronautical and space activities will best be served by vesting title to such facilities in the grantee. Accordingly, title to the facilities constructed with the funds provided under this Grant is vested in Leland Stanford Junior University. The subject Grant is made in contemplation of the potential effect of the new facilities in stimulating the growth of space related research at Stanford in the manner outlined in this memorandum and the University's proposal.

It is expressly understood that no charge will be made by Stanford to any agency of the United States' respecting the use of such facilities in connection with any Government sponsored research.

It is further understood that Stanford will, in the expansion of its research program, continue to make every effort to bring all of the various applicable medical, scientific and engineering disciplines to bear on the problems of biomedical instrumentation and space biology.

- 3 -In addition, Stanford will undertake, in an energetic and organized manner, to create a broadly based, multidisciplinary team to explore mechanisms whereby the progress achieved within biomedical instrumentation in particular, and space science and technology in general, may be fed into the industries and segments of the economy with which Stanford normally has close relations. This team will be composed initially of competent staff numbers of the University and may later be expanded to include scholars from other universities and institutes and thereby broaden the base of the group. Research is to be encouraged on ways and means to expand the search for practical applications on both a regional and national basis. Furthermore, the University will undertake to make the medical and scientific community, as well as the industrial and business communities aware of new opportunities for application of specific developments or processes stemming from the space program. J.E. Wallace Sterling OF POOR QUALITY ATTACHMENT 22

Grant Establishes Biomedical

Biomedical Instrumentation Lab

Gordon P. Smith Elected President Of Hospital's Board of Directors

Gordon P. Smith was elected president of the Hospital Center's Board of Directors at the Sept. 26 Board meeting, succeeding William R. Hewlett, Board president since 1958.

Mr. Hewlett, executive vice president of Hewlett-Packard Co., Palo Alto electronics firm, declined reappointment because of the press of business activities. He had been a member of the Board since its inception in 1956.

Mr. Smith, who was first appointed to the (Continued on Page 2)



OUTGOING PRESIDENT William R. Hewlett, right, receives a farewell gift from Gordon P. Smith, incoming president of the Hospital Board. Gift is desk set.

The National Aeronautics and Space Administration (NASA) has announced a \$535,000 grant to establish a biomedical instrumentation laboratory at the Medical Center.

The new facilities will permit a fourfold expansion of research efforts at the Medical School in the fields of space biology and biomedical instrumentation. Investigators hope to design devices which can detect life -- if any -- on Mars and other planets. They also anticipate that the techniques they develop may have broader applications in the fields of biology and medicine.

Research in this field is already under way in the Medical School under a three-year \$380,640 grant from NASA. It is being directed by Dr. Joshua Lederberg, executive head of the Department of Genetics. Associated with Dr. Lederberg and responsible for the instrumentation aspects of the program will be Dr. R. Elliott Levinthal, formerly president of Levinthal Electronics Products, Inc., and now a research associate in genetics.

The biomedical laboratory will be located in the new \$5.5 million Clinical Sciences Building, which is expected to be ready for occupancy in 1964. Instrumentation laboratories and shops will be on the ground floor; biological applications will be studied on the third floor in an area adjacent to the \$1 million Lt. Joseph P. Kennedy, Jr., Laboratories for Molecular Medicine.

The NASA-provided facilities will occupy approximately 10,000 square feet in the building. It is expected that cooperative research programs will be set up with other scientists throughout the Medical School.

The grant is one of the first research facilities grants ever to be authorized by NASA. Other grants, announced simultaneously in Washington, D.C., on Sept. 22, went to the University of California, (Continued on Page 2)

Hewlett, a Member Since 1956, Is Replaced by H. Edwin Robison

(Continued from Page 1)

Board in 1960, was reappointed by the City of Palo Alto for a three-year term ending in 1965. A management consultant, Mr. Smith is a partner in the national firm of Ecoz, Allen and Hamilton. He is the director of his firm's consulting operations throughout the West in the fields of public administration, hospitals and medical centers, educational institutions, industrial development and metropolitan area planning.

Robert H. Klein, who has been reappointed for a three-year term by Stanford University, was elected vice president. Mrs. Edward H. Heller, the first woman appointed to the Board, now becomes the first woman efficer. She was elected secretary-treas-

Stanford selected H. Edwin Robison to re-



place Mr. Hewlett. The director of the Economics Division at the Stanford Research Institute, Mr. Robison holds a threeyear appointment.

Since joining SRI in 1953, Mr. Robison has been engaged in a number of economics activities including area development, transportation, and industrial organization studies, as well as re-

search administration. He has served as an economics consultant in India and holds the Legion of Merit with Oak Leaf Cluster for his work in military government in Ja-

Theodore K. Strong, representing Palo Alt, was reappointed for a three-year term.

Stanford has not yet named the person to creed James B. DePrau, who also resigned ecause of business activities.

At the annual meeting of the owners held ast before the Board meeting, Mr. Hewlett raised Mr. DuPrau for his many contributons during his six years on the Board.

Of Mr. Hewlett, Mr. Smith said: "Under for stewardship we personally feel outtending accomplishments have been made by the Hospital Center."

Laboratory Will Provide Space For Cooperative Research Study

(Continued from Page 1)

Berkeley, University of Chicago, Rensselaer Polytechnic Institute and the State University of Iowa.

Dr. Levinthal said that it is important for a biomedical engineering program to be located within the Medical School. This is because staff members with technical competence in the physical sciences must be on hand to accelerate the response of biomedical specialists to advances in physics and engineering.

Close association between biomedical people and those in the physical sciences is necessary, he said, so that they can develop a common language and effectively exchange ideas. And only with this association can physical scientists come to appreciate the functional subtleties and structural complexities of living organisms.

Promotion Announced for Simmons; Jennison Is Appointed to Faculty

Dr. F. Blair Simmons, instructor in surgery (otorhinolaryngology), has been promoted to assistant professor effective Sept. 1.

Dr. Simmons, who first came to the Medical School as a resident in 1959, was named instructor in January of this year.

Dr. M. Harry Jennison has been named assistant professor of pediatrics. He was a clinical assistant professor in pediatrics, a physician with the Palo Alto Medical Clinic and school medical consultant for the Palo Alto Unified School District.

Appointed visiting professor of virology in the Fleischmann Laboratories of Medical Sciences for the autumn quarter is Dr. Harry Rubin. He is professor of virology, University of California, Berkeley.

A Doctor's Dining Room Is Setup in Cafeteria

A doctor's dining room of a modest sort was established in the Cafeteria on Sept. 15.

From noon to 1 p.m. each day the alcove near the main entrance will be set aside for physicians.

-Unclassified Ads-

FOR SALE: 1959 Morris Minor convertible; dependable, economical, about \$500. Owner going abroad. Call Susan Gibian, Exr. 5039 from 9 A.M. to 5 P.M.

STANFORD UNIVERSITY

OFFICE MEMCRANDUM

STANFORD UNIVERSITY .

OFFICE MEMORANDUM .

STANFORD

DATE: August 3, 1966

To : Robert G. Lindee

Ass't Dean Medical Affairs

From : Elliott C. Levinthal

Genetics

SUBJECT:

Possible response to your memo of June 13, 1966, addressed to Dr. Joshua Lederberg concerning NASA-Stanford basic agreement

Prior to the move into the new facilities, which has just recently taken place, we have taken some initial steps to implement the requirements of the final paragraph of the agreement.

The first step in broadening the interests of the scientific community in the problems of biomedical instrumentation and space biology is to make them aware of the relevance of this activity to their special academic disciplines. This approach has so far been very successful and has yielded some specific results.

We initiated discussion with Professor Stryer of the Department of Biochemistry about our interest in fluorescent assays as functional tests for the presence of life on Mars. This in turn led to collaboration with him in instrumentation development for nanosecond phospherimetry applied to problems of binding sites of myoglobin and hemoglobin. From this followed development work on a fluorgenic substrate that could function for a broad array of enzymes and which would be applicable both to the problems of exobiology and terrestial biology. Professor Stryer is now a principal investigator under a separate grant with the OSSA.

Our interests in application of mass spectrometry to space biology has led to collaboration with Professor Djerassi of the Department of Chemistry and his work on the analysis of natural products. We have held meetings with virtually every mass spectrometer manufacturer to make them aware of the specific developments we have completed, the objectives of our program, and its relevance to future instrumentation of this kind for biology and exobiology.

The group developing gas chromatograph resolving techniques to exploit the significance of optical activity as a sign of life has worked with the neurophysiologists to utilize the same techniques to elucidate problems in brain research.

The NASA research group collaborated and supported the Mechanical Pesign division in developing their interests in space technology. They now have established a direct relationship with Ames Research Center.

ORIGINAL PAGE IS POOR QUALITY

August 3, 1966

Space Science Board
NASA. This brought
on the problems of
ne recent publication
a group is to the
to the Automated
This effort has

Mr. Robert Lindee

Stanford hosted the Exobiology Study initiated by the Space Science Board of the National Academy of Sciences at the request of NASA. This brought together scholars from all over the country to focus on the problems of biology and the exploration of Mars and resulted in the recent publication of a two volume report.

The greatest commitment of the NASA supported research group is to the development of computer managed instruments relevant to the Automated Biological Laboratory (ABL) for planetary exploration. This effort has been one of the major stimuli in the development of the ACME (Advanced Computers for Medical Research) program which has now received NIH funding. The NASA group served to aid existing computer users in the medical school, initiate new uses, and through its interaction with the Computer Science Department and their general interest in artificial intelligence promoted their specific interest in the problem of computer analysis of organic structures via mass spectra, an issue crucial to exobiology in particular and biology in general, and in time-shared computer systems for medical research.

In developing the ACME program there has been a mutually beneficial dialogue with segments of industry important to space and computer technology. It has led specifically to IBM's development of equipment to interface efficiently between time-shared facilities and medical research activities.

21-

The following list of members of the ACME policy committee indicates the broadly based multidisciplinary team involved in this program. Because of this, its close connection with the NASA research activities and the interrelationship of computers to the next generation of biomedical instrumentation it is thought that this group could generate a specific proposal to NASA for further implementation of this aspect of the memorandum of understanding.

Dean Robert J. Glaser (ex-officio)

Professor Lincoln Moses (Statistics and Preventive Medicine)

Professor John W. Bellville (Anesthesia)

Professor Lubert Stryer (Biochemistry)

Professor Keith Killam (Pharmacology)

Professor Frank Morrell (Neurology)

Professor Edward Feigenbaum (Director, Stanford Computation Center)

Professor Joshua Ledergerg (Genetics), Chairman

ECL: jd

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NATIONAL AERONAUTICS AND SPACE ADMINISTRATION WASHINGTON, D.C. 20546

IN REPLY REFER TO

October 18, 1966

COT 2 II 1966 OFFICE OF THE DEAN

Dean Joseph M. Pettit College of Engineering Stanford University Stanford, California

Dear Dean Pettit:

The purpose of this letter is to let you know some of the things we would like to discuss during the visit of Mr. Webb and the other NASA representatives and advisors to Stanford on Saturday, October 29, 1966. As I indicated on the 'phone, we expect to arrive at 10:30 a.m. and remain through luncheon.

The principal purpose of our visit will be three-fold:

a. To review the manner in which the University is implementing the Memorandums of Understanding which have been signed by President Sterling and Mr. Webb in connection with the research facilities grants;

 To explore the impact of NASA upon the activities of the university, and

c. To exchange views on the role of the modern university in the space age.

Of these three objectives the first and third are probably the most important. We are interested in exploring the impact of NASA supported activities at the University but not in the context of detailed reviews of individual projects or programs unless they are activities that have been undertaken which might be considered specifically responsive to the first or third objective. Certainly we feel it important that a number of university people participating directly in NASA activities be included, but we are principally concerned with observing the impact that such studies may be having on the development of the University. We are particularly interested in learning of the University's response to the ideas set forth in the Momorandum of Understanding. In this regard I would like specifically to cite the last two paragraphs of the most recent Memorandum:



"The National Aeronautics and Space Administration is particularly desirous that the environment in which space research is conducted will be characterized by multidisciplinary efforts which draw upon creative minds from various branches of the sciences, technology, commerce and the arts. It is understood that Stanford will, in the expansion of the research program, continue to make every effort to bring all of the various applicable scientific and engineering disciplines to bear on appropriate problems associated with the space effort. The Stanford Program will be carried out in close coordination with its companion effort in medical instrumentation and space biology which has received substantial financial support from NASA as well as other space related work in progress or planned at the University. The research team will be composed of competent staff members of the University, expanded, as appropriate, by the inclusion of scholars from other universities and institutes to broaden the base of the group.

"In the prosecution of this program Stanford will undertake, in an energetic and organized manner, to explore mechanisms whereby the progress achieved in research may best be fed into the industries and segments of the economy (with which Stanford normally has close relations). Research is to be encouraged on ways and means to expand the search for practical applications on both a regional and national basis. In particular, the University will undertake to acquaint the scientific community, as well as the industrial and business communities, with new opportunities for application of specific developments or processes stemming from the space program."

Consideration of these questions naturally leads to the third purpose of our visit, namely the exchange of views on the role of the modern university in the space age. In view of the increasing role that science and technology are playing in our way of life and the potential contributions that they can make, there is a growing feeling in many quarters that the University should have an increasing role in interpreting science and technology for the common good. Since the space program is at the very cutting edge of science and technology, we are interested in examining the university's ideas and views in this area.

In summary, because of the commitment of Stanford University to the space program and the wisdom that has accumulated within the University over the years as one of the nation's outstanding educational institutions, we are interested in examining a number of rather broad issues, such as the growing

ORIGINAL PAGE IS OF POOR QUALITY role of science and technology and how to accommodate it in the modern university, the desirability of the multidisciplinary approach, and the desirability of increasing university effectiveness in the area of public responsibility. While we would like to discuss these issues broadly, we will, of course, have in mind the more parochial interest of how to improve government-university-industry teamwork and in particular, NASA-university teamwork.

We regret that President Sterling and possibly yourself will be unable to participate in these discussions. We are looking forward to meeting with Drs. Terman, Heffner, Lederberg, Rambo, and Hoff, and other members of the faculty and administration who are not only interested but can contribute to the discussions.

This year Mr. Webb has had a group headed by Dr. William Hagerty, President of the Drexel Institute of Technology and a consultant to the Administrator, and comprised of Dr. Raymond Bisplinghoff, Special Assistant to the Administrator, Mr. Breene Kerr, Assistant Administrator for Policy Analysis and myself, visiting a selected group of institutions for similar discussions. Dr. Dael Wolfle, Executive Officer of the American Association for the Advancement of Science and consultant to the Administrator has accompanied this group. It is expected that all of this group, with the possible exception of Dr. Bisplinghoff, will accompany Mr. Webb on the visit to Stanford.

Dr. Louis Mayo, Vice President for Advanced Policy Studies at the George Washington University, who is heading up the program of policy studies that has been initiated at GWU under NASA sponsorship, will also be in the party. There also will be several other NASA personnel and consultants in the NASA party who will be on the West Coast for the Berkeley activities, and it is possible that we would like to bring one or more of these men over to Stanford on Saturday. In all I expect the NASA group may be as large as 10 or 12.

I hope that this will be helpful to you in making the necessary arrangements for Mr. Webb's visit. I will keep you, or whoever will be in charge in your absence, advised of any changes in our plans.

Sincerely yours,

T.L.K.Smull Director.

Office of Gran ts

and Research Contracts

Tebruary 6, 1967

Mr. James E. Webb Administrator Mational Aeronautics and Space Administration Washington, D. C. 20546

Coar Mr. Webbi

Tam pleased to report on the accomplishments in specific areas of Stanford University School of Medicine since the signing of the Memorandum of Understanding Detween MASA and the University Concerning Research Facilities Grant MasG(7):2-52. You will recall that this agreement is in support of work performed in the Just uncertain Research Laboratory, which was formerly called the Medical Instrumentation and Exobiology Laboratories.

One of the most apparent—and necessary—accomplishments to date has been completion of the new 46 million Clinical Sciences Research Building, in which the Instrumentation Research Laboratory is located. Dr. Joshua Lederberg, 1958 winner of the Mobel Prize in Medicine and a Stanford University School of Medicine professor of genetics and biology, plays a leading role in the successful functioning of the new research building. As Enscutive Head of the Department of Genetics, had increasenable for research programs in neurobiology, coll genetics and biomedical instrumentation. He is also Director of the Lt. Joseph P. Kennedy, Jr. Laboratories for Molecular Medicine, which are located in the research building and are used by the Departments of Genetics, Pediatrics, Gynecology and Obstetrics, and the Meurology Division of the Department of Medicine. Dr. Lederberg's dual directorship makes possible the coordination of the various research programs.

An you know, the MASA grant of \$535,000.00 in 1962 made it possible to expand to the Instrumentation Research Laboratory. Dr. Elliott C. Levinthal, a senior research associate in the Department of Genetics, was appointed director of the laboratory. With this improved facility, our scientists and medical students have been able to pursue research in the fields of bicmedical instrumentation and exoblology with even greater momentum.

As anticipated, the convenient location of the Instrumentation Risearch Laboratory in relation to other compus facilities is promoting a definite increase in cross-fertilization of ideas and research. For example, Dr. Lederberg and his associates in the Department of Genetics have been interested in fluorescent assays as functional tests for the presence of life on Mars. They discussed this interest with members of the Department of Biochemistry, and as a result, the two departments collaborated in instrumentation for nanosecond phespherimetry applied to problems of binding sites of myoglobin and hemcglobin. This collaboration led to development work on a fluorescale substrate that could function for a broad array of enzymes and could be applicable, also, to problems of exchiology and terrestrial biology. Dr. Lubert Stryer, associate professor of biochemistry, is now a principal investigator under a separate grant from the Office of Space Sciences and Applications (OSSA).

There are many other examples of inter-disciplinary effort growing out of the NASA grant. For example, the Department of Cenetics is interested in the application of mass spectrometry to space biology. Their interest resulted in collaboration between the School of Medicine's Department of Cenetics and the Stanford University Department of Chemistry, with a new emphasis being placed on the analysis of natural products being done by Dr. Carl Djerasei, Stanford University professor of chemistry. Representatives of the two departments have met with virtually every mass spectrometer manufacturer to inform them of the specific developments, objectives and relevance of the program to future instrumentation of this kind for exobiology and biology.

The Pasteur Probe, an experimental approach applicable to Voyager Mars mission, uses gas chromatograph resolving techniques to exploit the significance of optical activity as a sign of life. The research group developing this has worked with Stanford neurophysiologists and utilized the usua techniques to elucidate problems in brain research.

Our NASA research group, consisting of approximately 25 people under Dr. Levinthal's direction, has collaborated with and supported the Hechanical Design Division of the Stanford University Department of Mechanical Engineering in developing their interests in space technology. The Mechanical Design Division has now established a direct and continuing relationship with Ames Research Center.

The greatest, present commitment of the NASA-supported research group is to the development of computer-manager instruments relevant to the Automated Biological Laboratory (ABL) for planetary exploration. This effort has been one of the major stimuli in the development of the Advanced Computer for Medical Research (ACME) program which has now received National Institutes of Health (NIH) funding. The NASA group sided existing computer users in the medical school to initiate new uses, and, through its interaction with the Computer Science Department and their general interest in artificial intelligence, promoted their specific interest in the problem of computer analysis of organic attructures via mass spectra, an issue crucial to exobiology in particular and biology in general, and in time-shared computer systems for medical research.

There is also the anticipated reciprocal benefit -- the utilization of the ACMS IBM/360-50 System in siding the conception and design of ABL missions. This relates to the Instrumentation Research Laboratory's current approach, which places greater emphasis on basic science and less on specific hardware implementation. For example, greater efforts are being expended on the basic science of the Pasteur Probe and the biological application of the techniques developed in connection with optical isomerium. The immediate objectives of the Laboratory's research groups are to descentrate the feasibility of a computer-managed laboratory in connection with its research in optical chemical specificity. This effort involves the use of mass opectrometry and gas chromatography as a primary, but not exclusive model.

The Space Science Board of the National Academy of Sciences, at NASA's request, initiated an Exobiology Study. This study was under the chairmanship of Dean Colin S. Pittendrigh of Princeton University and the co-chairmanship of Dr. Laderberg. Natings were held on the Stanford University campus and at the Rockefeller Institute in New York. These meetings began in the summer of 1964 and concluded with a session

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February 6, 1967

Mr. James E. Webb - page 3

in October, 1965. The working group accomplishing the study consisted of 36 people and represented a broad spectrum of scientific interests. Their findings have been published in a two volume book.

I should like to take this opportunity to express to you and your associates our appreciation and gratitude for your support. The results until now and the promise for the future amply justify the important part which NASA has had in making these possible. It is a part of which NASA can be proud.

In order to provide more insight into what we have been doing here at Stanford in the Instrumentation Research Laboratory, I am enclosing a report of progress and a list of publications. The enclosures include the construction and financial report on the Clinical Sciences Research Building; The Clinical Sciences Research Building brochure; the October, 1986, issue of Stanford M.D.; six releases from the Stanford Medical Center News Bureau, dated September 21, 1982, Jenuary 20, 1984, Jenuary 24, 1986, May 18, 1986, and May 28, 1986; four Medical Center Memos, dated March 28, 1982, October 10, 1981, May 10, 1986, and June 10, 1986; and a copy of the editorial appearing in the September 13, 1986, issue of Medical Forld News.

Under separate cover, I am sending to you floor plans marked to show the areas occupied by WASA and NIH in the Clinical Sciences Research Building. These plans indicate the besis for allocation of the costs.

I am sorry that you were unable to attend the formal dedication ceremonies for the Clinical Sciences Research Building on May 15, 1965. On this important occasion, Dr. Merbert E. Longenecker, president of Takane University, clearly emphasized in his speech on "Public and Private Partnership for Medical Progress" the need for closer cooperation between private universities and government agencies of which the MASA support at Stanford is a prime example.

I hope that you have time in the near future to visit Stanford egain, and that you will give us the opportunity of showing the Clinical Sciences Research Building and the Instrumentation Research Laboratory to you.

With best wishes.

Sincerely yours,

J. E. Wallace Sterling

bcc: Peter E. Pratt
John P. Clarkin
Hubert Heffner
Joshua Lederberg
Elliott C. Levinthal

An Instrumentation Crisis in Biology

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Joshua Lederberg
Professor of Genetics
Stanford University Medical School
Palo Alto, California
May, 1963

AN INSTRUMENTATION CRISIS IN BIOLOGY

Physiologists, especially students of the nervous system, have a long history of involvement with electronic instrumentation, many of the primary signals being already in electrical form. Biochemistry has made much less effective use of such instrumentation - laboratories like Britton Chance's being quite exceptional. In consequence, we face some of the most profound issues of biology, in molecular biology the sensitive detection of macro-molecules, and the specification of their ultrastructure and metabolism, with tools that are astonishingly primitive by the standards of instrumentation available in other fields. This is especially true of the range of optical instruments, which are slow, imprecise and require inordinately large samples of material compared to some plausibly attainable possibilities. A second area of data processing in which a great burden of manual effort can be lifted is particle-counting which, in one form or another, is the fundamental measurement in many aspects of biology (especially microbiology, cytology).

Finally, perhaps most exciting, an immense amount of information is still locked up in spectra (optical absorption, magnetic resonance, rotary dispersion, mass spectra) and similar fingerprints, which require the intensive development of the "man-computer symbiosis" for adequate resolution. While such successes as the structural analysis of myoglobin have had well-deserved attention, even they focus attention on the importance of further instrumentation development to solve such problems on a broader scale (each one should not be a tour-de-force, at least not after the first one.)

The inadequacy of current art in biochemical instrumentation was brought home to us in our efforts to meet the mission requirements of exobiological studies, but they are equally pertinent to present efforts in the terrestrial biochemical and microbiological laboratory, except of course we at least do have the traditional tools to work with meanwhile. We have been delighted at the opportunity created by NASA's programmatic and financial interest to try to contribute conjointly to exobiological and terrestrial needs in biological instrumentation.

Over the past three years, we have gradually been organizing an instrumentation group within the Genetics Department. Under Dr. Levinthal's immediate direction, its scope can be indicated by a present staff of three additional electrical engineers, (L. Hundley, H. Horn and N. Veizades), a physicist (M. Mandel), and for the exobiological work, a biochemist, (Dr. E Shneour). Two or three additional appointments are in prospect.

Some of the more directly mission-oriented work has been reasonably successful, for example the mechanical design an prototype construction of a "multivator", a compact laboratory for sampling and chemical analysis of Martian surface dust. On the other hand, as any experienced hand would have predicted, many of the "bright ideas" we have developed, either as requirements for instruments, or as their designs, have bogged down

when the construction and debugging of the devices took months instead of weeks. The worst result is perhaps the loss of interest in the original purpose of the design; the next worst is the reaction of hypercaution in deciding whether to go ahead with a given project.

The Instrumentation Crisis is thus deepened by the lack of flexibility that these considerations imply, and the practical attitude of disdain for preoccupation with instrumentation displayed by most members of the biochemical scientific fraternity.

Digital computation may help answer these needs in several ways. Precision can be improved in data-processing links as an inherent virtue of a digital system. Unfortunately the original data are generally in analog form, and the accuracy of analog-to-digital conversion will be a limiting factor. However, precision is also very often a signal-to-noise problem, and an ideal instrument should have the flexibility to allow accuracy to be purchased at the price of speed, in accordance with local needs - the memory capacity of the computer for averaging over time, and the use of correlation techniques to extract signals from noisy inputs, suggests the application of simple computer techniques to improve the utility (and to simplify the potential design) of such workaday instruments as the absorption spectrophotometer. Since many problems cannot escape the dilemma of requiring measurements of differences between larger values, this is no luxury. Probably more important is the construction of (at least) the prototypes of new instruments by programming a general-purpose computer to set up the control and signal-processing systems, instead of de novo construction.

Further, mechanical corrections to maintain linearity and stability play a large part in the design of most instruments - for example the slit-width control to maintain constant reference brightness with varying wavelength in a spectrophotometer, or the slide-wire bridge to obtain an intensity ratio in its output recorder, and these generally impose severe penalties in the complexity of design as well as the accuracy of the instrument. A fairly small memory and computing element would replace most of these expedients. It is likely, but not certain, that the specialized training needed to make some intelligent use of programming in instrument design is less than needed for hardware construction, a feature that would make innovation more widely accessible to other biochemists in a university environment. The computer is, of course, a much more complicated instrument than any of these, but it is intrinsically more accurate; it has programmable versatility, and most important it was designed and built once and for all.

Finally the course of <u>future</u> developments certainly seems to call for the preeminence of digital (or mixed) computation, and the emergence of more and more compact and reasonably priced systems. We have therefore determined to reorient our entire program towards the most efficient use of digital computers as on-line elements. Since our resources would still not justify capturing a larger machine, our opportunity to proceed along these lines have only recently opened up through two possibilities: (1) a time-sharing system, and (2) the LINC.

We do not know which of those affords the greater promise--probably each of them has a particular place, and we can hardly ignore the possibility of further interaction between them. Our special attributes for the evaluation of LINC then perhaps include (a) newcomers to extensive use of digital computation; we have no on-line experience at all, except insofar as some use of digital elements has appeared in our own instruments; (b) a team effort, intended to serve a variety of instrument needs, but with special emphasis on biochemical analysis (nucleic acids, proteins, enzyme assays); (c) a comparison of utility of LINC and of time-sharing in a variety of situations.

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The time-sharing system referred to is the special interest of Professor John McCarthy of the Stanford Faculty in Computer Sciences. Our laboratory will participate in a program he is designing to allow some few stations to share access to a PDP-1 computer, which will in turn be coupled to the IBM 7090 system now in operation. The PDP-1 is scheduled for delivery next month; within a few months thereafter we should have telephone or direct wire access to it from our own laboratory. The final configuration remains to be worked out; some buffering to mitigate the disturbance of interrupts is intended to make the system useful on line.

The current active projects reflect an over-conservative reaction to recent problems; I am, therefore, also appending a list of other items we would keenly like to be able to manage, and urgently hope to get into. Some tentative priority is given, but this will be tempered by the acquisition of some additional people, and by a reappraisal of the actual utility of LINC or the PDP-I time-sharing arrangements in each case.

CURRENT PROJECTS: ACTIVE CONSTRUCTION AND TESTING

Some additional details are given in appended descriptions:

Multivator. A device for the acquisition of samples of planetary dust, their distribution to individual compartments, programmed release of solvents and reagents, and the determination of enzymatic activity or growth by various photometric measurements (absorption, fluorescence, scattering, polarization, scintillation).

Mark III incorporates a flying-spot scanner to identify local areas of enzymogenic fluorescence, the local illumination enhancing signal/noise. The signal is expected to reflect localized incidence of microorganisms; the noise comes largely from the background fluorescence of the soil sample eluate and from spontaneous degradation of the fluorogenic substrates (e.g. fluorescein phosphate).

Videoscan Spectrometer. A fast scanning spectrophotometer based on the projection of a spectrogram on the target of a signal-storing image-forming television tube. This was primarily intended for microspectrometry as a means of searching for microbes in soil; this application will require the delivery of newly developed, UV-sensitive video tubes to complement Zeiss ultrafluor optics obtained with Caspersson's cooperation. The basic system has been completed, and is being tested by being installed in a model E analytical ultracentrifuge to allow online reading of absorption profiles (hopefully also absorption spectra at each stratum) of DNA in the course of pycnographic fractionation. As these centrifuge runs may require two to several days, the accurate determination of the profiles online should greatly shorten machine time; as the signals are already in electrical form, the computation of peaks, band decomposition, integration and linearity corrections, would all be facilitated.

The same video system can be and has been used for more general purposes mensitometry on photographic spectrograms, wherein it is being compared
with flying-spot video. It is also being set up for simplified framedifferencing, to allow the discrimination of moving targets: in our
application, the tracks of microbes with purposeful motion. Having spent
some years in "human" microscopy of individual bacteria, in connection with
the biochemical genetics of their flagella, I am looking forward to the
possibility of computer-assisted analysis of this primitive behavior.

The control system of the videoscan is a versatile one, especially to allow alternate scanning of a reference and sample field; then a line-interval delay allows the superimposition and comparison of sample/reference, giving an equivalent dual-beam mode. Plainly, most of this could have been quickly by-passed by exploiting a general purpose computer.

Colony Counter (Iconumerator). The most tedious operation in bacterial genetics is counting colonies of plates. We inherited an Iconumerator (a flying-spot pulse analyzer) of World War II vintage from the DuMont Laboratories

Colony Counter (cont)

and have been trying to make it work properly. This is difficult, apart from the limitations in its one-line logic.

The most serious problem is the reliability and resolution of the delay line, which stores information for common-mode rejection of a colony count at second encounter with the scan beam. We have been speculating about a major redesign and rebuilding with modern components; would very much prefer the course of simulating a new design and trying it out quite extensively before making a large commitment of time, effort and money. Quite possibly the whole issue can be evaded by other mechanical arrangements (a microbiological machine that disposes the inoculum in a linear array, so that counting can be done by counting pulses along a single axis).

Curve Reader and Analyzer. This is an elementary combination of XY-plotter and curve follower, and tape-recorder, synchronized to a small analog computer. Chart records are thus translated to signals on each of several channels, which can then be fed synchronously through the analog computer for analys's. It has been useful for elementary functional transformations, e.g. of recorded and published absorption spectra, and for instructional purposes.

POTENTIAL PROJECTS: ACTIVE CONSIDERATION, especially for digital enhancement

1. Precision Photometry; laboratory measurements

The straightforward enhancement of the performance of existing instruments and techniques may be the most elementary but rewarding application of computer techniques, using the principles already summarized. Spectrophotometry plays a key role in all our work on bacterial DNA; we would like to be able to measure more accurately in the range below o.l optical density unit. Even a very simple instrument in which reference information can be sampled and the signal time-averaged over some interval should be able to do this for us.

An analogous statement can be made for spectrofluorometry. Further, Dr. Lubert Stryer is soon joining the Biochemistry Department, and is especially interested in cooperating here towards the development of new analytical techniques which require the utmost detectivity in fluorescence measurements.

2. Depolarization of fluorescence; measurement of relaxation times.
One of the most promising simple methods for estimating molecular size of DNA and following it through thermal transitions is the measurement of relaxation times after electric orientation of the solution. For sensitive detection, we propose the use of fluors coupled to the polymer, and the use of polarized exciting light.

As relaxation times of hundreds of microseconds are anticipated, the entire control and analysis program should be compatible with the LINC's capabilities. This approach may have special promise for the detection of macromolecules in complex solutions for exobiological purposes, among others.

- 3. Optical rotatory dispersion plays a special role in long-range thinking about exobial detection for reasons we owe to Pasteur. Precision measurements, especially of highly absorbing materials like polynucleotides, are essentially a problem of extracting signal from noise. Our colleague, Professor Djerassi in the Chemistry Department, is especially interested in such instrumentation, and is a well-known authority on its utilization in chemical analysis.
- 4. Microscan mass spectrometry. This is the most ambitious project but may be the most hopeful in meeting urgent requirements at the focus of several interests in the department; genetic chemistry, neurobiology, and exobiology.

We propose to scan a specimen at (crude) electron microscope resolution with a high intensity beam along the lines of the electron probe micronalyzer. However, the scanned spot would be volatalized and the gas fed to the beam of a fast mass spectrometer (e.g. - the Bendix Time-of flight instrument) for mass analysis. At low mass numbers, and with

Microscan mass spectrometry (cont.)

various other tricks, it might be most useful to localize tritium at resolutions, perhaps even sensitivities, exceeding current autoradiography. (The LINC would play a special role in processing the spectral outputs for tape storage, selection and readback, either through the LINC or through another computer for even more detailed analysis. It could, of course, also furnish the principal control mechanisms.)

5. Microfluorometry. Dr. Boris Rotman, in this laboratory, devised a method for the detection of enzyme activity not only for single bacteria, but even single enzyme molecules, based on the microfluorometry of fluorescein released from a galactoside conjugate. He has since moved to another laboratory in the area, but we are maintaining a close collaboration. Much of this work requires a very laborious sizing of microdrops under the microscope, and then a determination of fluorescence, both of which should not be too difficult to instrument for mechanical operation.

As the analytical technique is mainly based on Poisson statistics, a large number of drops must be analysed, not to infinitesimal precision. This equipment would be of very great value to both of us in studying the role of DNA in instructing the cellular synthesis of enzymes.

ADDENDUM

Further extensions of computer technique in human biology and genetics.

In addition to laboratory experiments these studies of exprequire the analysis of large volumes of vital statistics and similar data. The indispensability of computers in tabulating information from such files is of course well understood. However, there are serious problems in the accessibility of computed data in this area, just as there are in experimental work. As important as are the obvious current applications of computers in data processing, we should not be content with the present system but work for more flexible means of experimental access to such data through "real time computation".

The small computer, like the LINC, or the time-sharing system, furnishes an answer to this need. Very extensive data files (up to 300,000 tab cards) can be stored on a single reel of magnetic tape which need require no more than five minutes to pass through the computer. A flexible system would allow this to be done under direct manual control and with immediate console display of computation on these data files. In most applications, except those that involve very extensive sorting and resorting, the actual computing time in a large computer like the IBM 7090 is relatively short compared to the time required for the input-output processes. With the appropriate organization of the computer facility, it should therefore be entirely feasible for the investigator to deal directly with the large data file in real time, while his attention is still concentrated on the problem at hand and in the formulation of new hypotheses for prompt testing. Only in this way can we really make the most effective use of the combination of human ingenuity and constructive imagination and the computer's capacity to undertake a humanly impossible task of drudgery in computation.

The most evident application of this approach in genetics should be in the analysis of vital statistics. In the experimental sciences the nearly analogous applications will involve searching through data accumulations like spectra and other physical properties, the experimenter forming generalizing hypothesis and using the computer to test them against the file.

Experienced insight into the relative roles of the human and machine components of these systems should be invaluable in the further development of artificial intelligence, the programming of machines to simulate as far as possible those human cognative processes that we can begin to understand. These generalizations of human intellectual capability, and their delegation to machines, continue to refine the strategic role of the human element and to give it increasing leverage in the solution of complex problems.

Proposed Supplement

to

National Aeronautics and Space Administration .

Grant NsG 81-60

for period

April 1, 1963 to March 31, 1964

Prepared by:

Professor Joshua Lederberg
Dr. Elliott C. Levinthal
Department of Genetics
Stanford University
School of Medicine
Palo Alto, California

Approved by:

This is a proposal requesting a supplement of \$132,000 covering the period April 1, 1963 to March 31, 1964, to our existing grant NsG 81-60. The purpose is to allow expansion of our exobiology activities and an orderly growth of the total instrumentation program to the level envisaged for the new laboratory space provided under the facilities grant NsG(F)-2.

This is necessary to meet the pressing requirements of the exobiology program generated by the specific interest in 1966 Mariner missions and is desirable for the general program, insuring a rapid utilization of the future laboratory space in the new Clinical Sciences building. It has been made possible by the Medical School making available 2000 square feet of additional net laboratory space for the activities of the laboratory, which is now named the Instrumentation Research Laboratory.

The present staff and facilities are completely engaged in work related to the exobiology program. It is particularly urgent that the instrumentation efforts related to Mariner missions be expanded as soon as possible. These efforts presently include collaboration with the Mechanical Engineering Department program under Professor Arnold, concerned with Multivator design and with the specific objective of a working model as soon as possible. In addition, in our own laboratories and shops, we are pursuing and wish to increase our efforts with regard to improved versions with higher sensitivities. In parallel with this is the development program, also in need of expansion, of biochemical assays to be carried out in the Multivator biochemical laboratory.

This is in addition to the laboratory program related to later possible missions which will involve video capabilities and thus be concerned with microscopy and U.V. spectroscopy.

Besides the laboratory program, we have started investigations into what might be termed theoretical exobiology. The initial efforts, which also need expansion, are to generate a matrix of signs of life of varying generality with regard to the postulates of the life system versus the degree of evolutionary development. The purpose here is to provide a critical basis for making a choice of life detection experiments and, hopefully, to generate new ideas for better experiments.

During the past several months new opportunities have presented themselves at every level of present activities as a result of a cooperative effort with the facilities and personnel of the Stanford Computation Center. We propose to enlarge this area of activity by the purchase of image-reading components for the time-shared PDP-1 computer to be installed. This will greatly enhance our ability to design possible imaging devices and to evaluate possibilities of logical analysis of video information. This purchase will, in addition to providing facilities specially suited to our needs, entitle the Instrumentation Laboratory to a year's time-shared use of the PDP-1 computer. For this purpose we are requesting \$25,000, of which approximately \$21,000 is for the purchase of image reading components and \$4,000 for programming and run time on the IBM 7090 system at the Stanford Computation Center.

We are also planning to submit a proposal to the LINC Evaluation Board to qualify for the allocation of a LINC computer. The Instrumentation Laboratory would then be in an unusual position to compare the effectiveness of these two concepts, time-sharing on a large computer system (the PDP-1/7090 complex) versus the full-time availability of a smaller one, the LINC, for a variety of problems in medical instrumentation, measurement and analysis. In view of the bandwidth limitations on the Mars-Earth channel, the importance of attaching a large degree of decision-making computer capability to the landing missions for life-detection and life-characterization is self-evident.

Professor John McCarthy of the Computer Sciences Department is particularly interested and experienced in such problems, and is cooperating with our investigation of them.

Collaborative interaction of the Instrumentation Research Laboratory with other Medical School research has been initiated, resulting in the funding of more than one program by another agency (NIH) and others under active discussion. We are proposing to increase this role of laying the initial groundwork of cooperative activities which will ultimately be a significant activity of this laboratory.

We propose to increase our present level of expenditures starting in April so that by the end of December we will be operating on about a \$27,000 monthly rate. To carry out this program until March 31, 1964 will therefore require \$83,000 in additional funds for the operating budget. In addition, to meet the special requirements of model building related to the Mariner missions, we would like funds and the necessary authorization to purchase \$25,000 worth of machine tools. For the computer facilities and services discussed above we require \$25,000. Thus the total request for additional funds is \$132,000. The program for the following three year period, extending to 31 March, 1967, is now being prepared and will shortly be submitted. It is anticipated that this program will involve an expenditure of approximately \$500,000 per annum and will be compatible with the space provided by the NASA grant and the functional scope of the Instrumentation Research Laboratory program envisaged.

This expansion will result in the following complement of senior staff for the laboratory by the end of the year:

Principal Investigator

Program Director

Engineering Group

Biochemistry Group

Physics/Mathematics Group

Professor J. Lederberg

Dr. E. Levinthal

Harrison Horn Lee Hundley Nicholas Veizades

Dr. Elie Shneour Jerry Lundstrom < Position to be filled >

Dr. M. Mandel < Position to be filled >

Supplementary Budget

Salaries	\$52,000
Overhead (43%)	22,360
Payroll charges (7%)	3,640
	\$78,000
Supplies	4,000
Machine Tools	25,000
Computer Equipment	21,000
Computer Services and Programming	4,000
Total Supplement	\$132,000

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Stanford Winter 1963 MORE 1963 MORE 1963

THE MULTIVATOR: Martian Life Detector

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Stanford winter 1963 Today

MAN IS LOOKING to other planets for the answer to the most fundamental and perplexing research problem of all: how did life begin? Stanford geneticists and engineers are working together on one part of the search. They are experimenting with a life detection instrument which, landed 50 million miles away on Mars, could radio back evidence of microbial existence.

In LIFE BEYOND THE EARTH

Joshua Lederberg tells of the new science, exobiology. He pioneered the field and gave it its name.

PAYLOAD TO MARS sets the matrix for space life missions. Elliott Levinthal-physicist who has become something of a chemist and biologist as well-is the author.

THE MULTIVATOR (shown on the cover) is one choice of a detection system being developed in Stanford laboratories.

STANFORD TODAY

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"THE INSISTENT QUESTION"

THE BIOLOGIST is a homely and practical-minded person, who is little given to over-refined logic and debate, but much given to observation and experiment. His laboratory tells him what a precarious and fragile thing life is, how material and condition-ruled and circumscribed a living creature is. But his wife and child and his own consciousness tell him much more, how immeasurably much more, there is in life than he learns in his laboratory. It is this extra-laboratory observation and realization of the possibilities and actualities of human life that make it, even to the biologist, the vivid, many-colored, suggestive, and thrilling thing it isthe thing so full of occasionally realized great moments and of glimpses of infinitely great possibilities, that sometimes it seems all mystery, all something more than of this world, and hence all something quite hopeless to study by the methods of his science, indeed quite hopeless even profitably to wonder about. Why not take it and make the most of it?

And then comes the insistent question: Ah, how make the most of it? And he becomes again the patient, struggling student of biology, the student of the laws or conditions of life.

VERNON L. KELLC'S, Stanford Professor of Entomology, 1894–1920, in *The Atlantic Monthly*, June 1921.

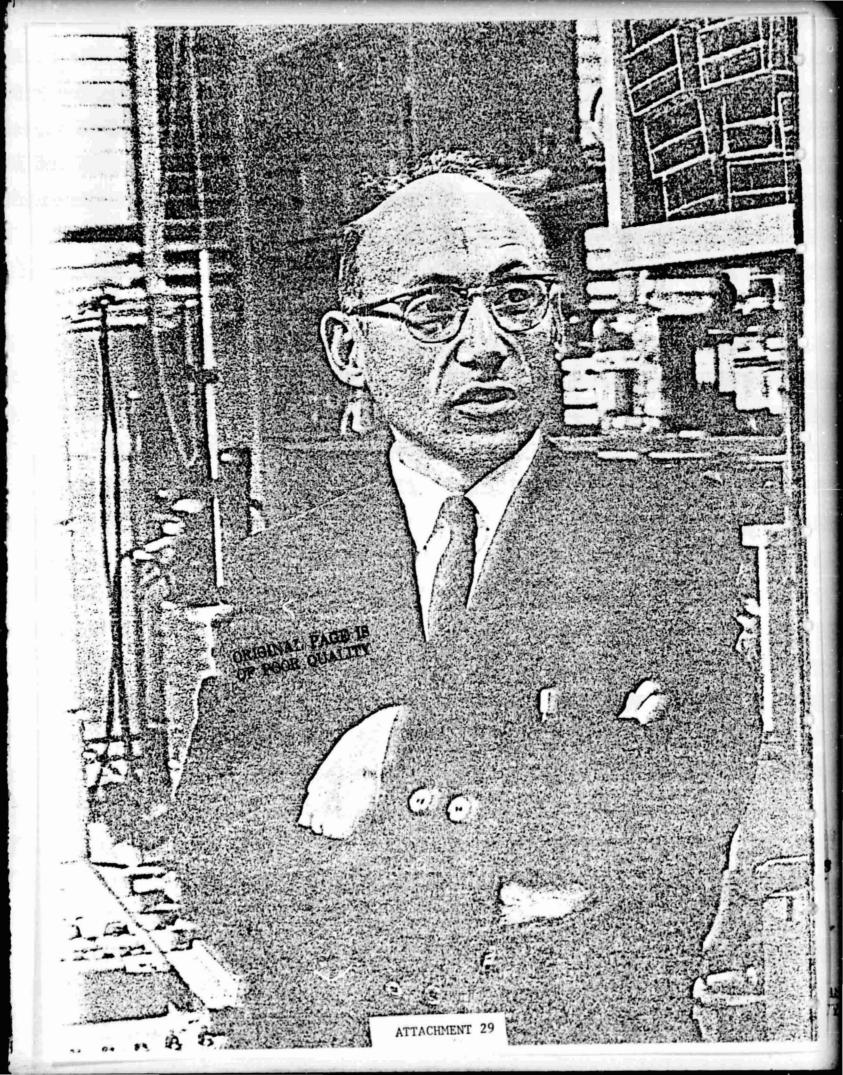
LIFE BEYOND THE EARTH

by JOSHUA LEDERBERG

TO THE HISTORIAN looking backward from future time, this century will be memorable for a number of peaks in the evolution of human culture: the concentration of national power in global conflict, the shrinking of the globe by air transport and wireless communication, the exploitation of nuclear energy, the technological revolution of computers and automation, the reunification of the sciences, the unraveling of the physical mechanism of life. All of these movements have a common focus in the exploration of space, the millionfold extension of human activity from the earth's crust to the reaches of the solar system. The same power and resources that can count down the survival of civilized man can also light his noblest aims. What do we seek in space? Not astronomical

little. The journey does give us two unique rewards: a perspective on our own planet and a prospect on other worlds. The first pioneering steps in space-the orbiting satellites that can analyze our atmosphere, show our weather, speed communications, help navigation, or warn of global dangers-are already doing their useful tasks and begin to show their merits in advancing scientific knowledge of the earth. Now, as our vehicles become more powerful, we must measure our reach and attend to our objectives in exploring the other celestial bodies. Among these objectives, the study of life beyond the earth, which we term "exobiology," is the most subtle and demanding, for it insists: "Know thyself."

numbers. Emptiness multiplied is at most very



DIOLOGY has experienced an amazing development as a scientific discipline, particularly in the biochemical understanding of the mechanism of life. But its domain has been limited to the thin shell of our own planet, to the way in which one spark of life has illuminated one speck in the cosmos. By contrast, the basic laws of physics are derived from the motion of the stars, and we know the scope of chemistry from spectral analysis of the light emitted by stars and galaxies at the boundaries of the observable universe. Biology has no such grand system. There is perhaps only one principle which we can confidently expect to have universal application. This is Darwin's principle of evolution, the magnificent process which explains how the complexity of amoeba or the grandeur of Sequoia sempervirens could have materialized.

The evolution of life on Earth, we now know,

occurred in three stages:

Chemogeny, the production of complex organic compounds by a variety of nonreplicative mechanisms—the primitive aggregation of matter, photochemistry of isolated atmospheres, thermal, inorganic-catalyzed, and spontaneous reactions of previously formed reagents.

Biogeny, the replication of a specifically ordered polymer (DNA) which specifies the sequence of its own replicates, and of RNA and proteins, from which cells and organisms are fashioned. Random error in replication and natural selection of the consequences result in the panoply of terrestrial life.

Cognogeny, the evolution of the mechanisms of perception, computation, and symbolic expression, which are the unique conditions for interpersonal communication, so that, from this,

tradition can accumulate.

Despite their outward variety, the central components of all organisms are the same: their genetic material consists of nucleic acids, most of their structure consists of proteins. We are beginning to understand how the chemical properties of these substances underlie their function in the cell. But all cells have the same general composition. The boiled-down residues of the beef muscle would be hard to tell from the mushroom sauce; the nucleus of the human nerve cell from the virus that might attack it.

The conception of the central unity of terrestrial life has quickened the search for the origins of life. If we knew how specific proteins and

nucleic acids first appeared on the earth, we would have most of what we need to understand the further development of life. In the world now, proteins and nucleic acids are produced only as manifestations of life, as copies of what had evolved before. But how did this come about spontaneously, without pre-existing cell or brain to guide it? Thirty years ago this was regarded by some as a problem that might never be solved, as something beyond the reach of science. Today it leads to fundamental questions of exobiology. Are nucleic acids the only substances that can function in any heredity or are they merely the ones that the path of earthly life has encountered? Are proteins, chains of just 20 amino acids, the only way of building up cell structures, or the accidental result of early chemical evolution on earth?

These questions might be answered in two ways. Presumptuous man might mimic primitive life by imitating Nature, furnishing substitute compounds. More humbly, he might ask Nature the outcome of its own experiments at life, as they might be manifest on other globes

in the solar system.

F ALL the planetary conditions, the abundance of water may be the most pertinent condition for the distribution of life as it is on Earth. All living cells contain far more water than any other component. Water plays many roles in the economy of the cell, but above all it is the indispensable solvent. The intricate work of the cell requires the ready intermingling of many kinds of molecules. This could only occur in solution, and, if not in water, the only obvious alternative, and only at very low temperatures, is ammonia or a similar liquefiable gas. Many other substances are vitally important to our own existence-for example, the oxygen in the air we breathe. But our own dependence on air should not exaggerate the generality of its importance. The vegetable kingdom and many simpler animals can survive without an external supply of oxygen, and even quite complex forms of life should be able to thrive without it, though they might replace the more efficient burning of foodstuffs with fermentation. Poisonous gases, like formaldehyde or carbon monoxide, in a planetary atmosphere might preclude human life, but man is not the measure of all things.



AUGUST 10

ORANGE August 22
Showing opposite hemispheres

RED





SEPTEMBER 11

ORANGE

PTEMBER 11 BLU

MARS IN 1956—Photographic images of Mars taken in different months with 60-inch Mt. Wilson telescope filtered to show the planet in red, orange, and blue light. The atmospheric haze in September has obscured much of the detail visible in August. Little but the polar cap, shown uppermost in each image, is visible in the blue light. (California Institute of Technology—Mt. Wilson Observatory photograph)

HE MOON, Venus, Mars, and Jupiter are the celestial bodies nearest Earth and almost certainly will be on the itinerary of spacecraft in the decade ahead. Mercury is too close to the sun and Saturn, Uranus, Neptune, and Pluto too far from it to be on any early timetable.

The moon is handiest, but we are already positive that it is not quite large enough for its gravity to hold an atmosphere, and any free water on the surface would long since have evaporated into space. Some of these atoms will even have distilled over to the earth. We should still expect some traces of ice on the moon—condensed in sunless crevices—but these cold spots make it even more certain that the surface has no water, as it has no air, no weather of any kind, no present life. The moon thus is a marvelous relic on which to trace the primeval formation of the solar system. Its features have not been subjected to the continual metamorphosis we see on

weather-beaten Earth, and it is a target for the relentless impact of meteorites, particles ranging in size from single atoms to small planetoids orbiting in space until they happen to collide with the moon or a planet or are drawn into the sun. These meteorites have rained on its surface for a billion years unhindered by the atmosphere that fires their passage to the earth. The fossil moon therefore has its own sediments, a timeless record of cosmic history in the accumulated deposits of these materials collected from interplanetary space.

It would be of exceptional interest for cosmic biology to know whether meteorites can bridge the void from one planet to another. The moon's surface is almost the only place where we might find direct evidence of such an outflow from the earth. If the traces can be proven on the moon, we then could calculate that all the planets had interchanged fragments, perhaps even planetary systems of one star with another. This interchange would be much less than between the earth and its moon, and we might have no hope of finding direct evidence of it. But the arrival, just once in geological time, of a single fragment bearing a living spore would have immense potentialities for the future of a planet. The original purity of the moon's surface is thus an important scientific asset that should be conserved until we can plan our search for earthly traces on it.

not only to its relative nearness and largeness, but also to the high reflectivity of
layers of clouds which completely envelop it and
which have, so far, prevented astronomers from
seeing any of its surface details. Scientists had
speculated that the surface might be relatively
dark and cold, despite the closeness of the sun,
because of the cloud shield. Now it is known that
the surface is hot, attributable to the "greenhouse
effect" of the high content of carbon dioxide in
the atmosphere. Measurements by radiation
methods indicate a surface temperature of about
400° F., and data gathered in the 1962 Mariner
fly-by may raise this estimate beyond 600° F.—a

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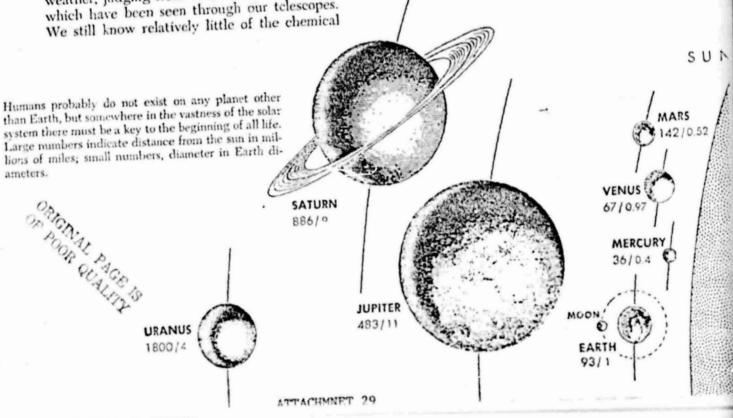
sizzling oven in which neither liquid water nor typical organic molecules could long survive.

If these measurements were confirmed by direct access of experimental instruments (i.e., a thermometer) we would have no basis for pursuing a search for life in familiar form. But, while it is hard to doubt the indicated temperatures, it is less certain where they occur. Most of the moisture on the planet will be found in its higher atmosphere, where more moderate temperatures may also prevail. Perhaps, then, the place to look for any life on Venus is not on its searing surface but in the clouds; in any case we must know much more about Venus's upper atmosphere before we start taxing its real estate. It is true, by way of analogy, that our own atmosphere bears a surprising amount of life-the birds and insects and, more pertinently, a wide variety of vegetable and microbial spores-though we doubt that any forms live out their full cycles in the air.

ARS, being somewhat farther from the sun, may have a temperature regime slightly chillier than Earth's, but it might likewise have retained a larger fraction of water and other important volatile material. On the other hand, Mars has only about a tenth of Earth's gravity and has been able to retain only a thin atmosphere, most of the lighter gases already having escaped. What remains might be compared to our own atmosphere at a height of at least 40,000 feet. This is far too thin for human breath but enough to give the planet a turbulent weather, judging from the massive dust storms which have been seen through our telescopes. We still know relatively little of the chemical

composition of this thin atmosphere. Of the 50-million-odd miles to the planet, the first 50 miles —Earth's atmosphere above the telescope—give us the most trouble. The only gas of which we have any definite knowledge in the Mars atmosphere is carbon dioxide; it is generally inferred that there is very little, if any, oxygen or water vapor, and that nitrogen makes up the main bulk of the gas. As a place for human habitation, even exploration, Mars would be considerably less congenial to human access and modification than the bottom of our oceans. But it is the abundance of water that must dominate our evaluation of the planet as a home for adaptable forms of life.

What then of water? Spectrograms have given direct evidence of only traces of it in the Mars atmosphere, and we cannot find seas of liquid water on the surface, but the frost is there to see as polar caps which wax and wane during the local winter and summer. The details of this weather circulation, how much water may be trapped in the soil of the temperate zones, are the key to the Martian mystery. Many astronomers have commented on the changing extent and color of dark patches that appear in these zones and particularly behind the receding polar caps in spring. These observations, reminiscent of a vegetation cycle, have been bolstered by Sinton's spectroscopic measurements through the Palomar telescope; the infrared color of the dark



patches corresponds to that of a layer of organic material. But this still leaves some doubts. Carbonaceous colors could mean life, but could also come from some inanimate process. Dollfus has seen the granularity of the Mars surface change with the seasons, also just as if this were the growth and decay of small plants. Taken together, these studies give little encouragement for the development of a Martian life as rich as Earth's, but they do not rule out a marginal biology whose urgent need is the finding and retention of water.

the sun, is a cold planet. Its chemistry is altogether unlike that of the group of rocky planets we have just discussed—the earth and its neighbors have only the dregs of the volatile material which comprises most of the universe, most of this having been distilled off by the sun's energy. Jupiter is immensely larger than Earth, but its specific gravity is very low and it must be composed mainly of condensed liquors and ices of compounds of hydrogen, oxygen, carbon, and nitrogen. These substances are the very raw material of the evolution of life, on the one hand, and of the evolution of the universe, on the other.

It will take some presumption to plan the direct approach to this huge and mysterious planet. Its gravity, three times that of Earth, must be resisted by the landing vehicle. The low density of the surface material will call for bulky, buoyant structures. The depth of the atmosphere and the violence of its electrical storms (already heard in our radio receivers) will complicate the task of communication which distance already makes difficult.

The moon, Venus, Mars, and Jupiter-these

make up Earth's "front yard' in the solar system (with human arrogance or humor, we call this neighborhood "outer space"). Of these globes Mars presents the highest probability of sustaining forms of life comparable in any way to those we recognize on Earth, and it is therefore the primary target of our research in exobiology.

For the near future, the exobiologist or his agent cannot lift his own fragile, demanding body very far from home. But unmanned vehicles have three important purposes: They can lift telescopes into orbit past the murkiness of our atmosphere; they can carry instruments to the planets for radio communication back to Earth; they might bring back samples for detailed study in our own laboratories, although it may take another decade to build the staged rockets needed to return even a minute sample from Mars.

Moving a telescope outside our atmosphere gives larger advantages than might be supposed for a journey of only a few hundred miles. Different gases, oxygen, carbon dioxide, water vapor, which are so important in the analysis of planetary atmospheres for signs of earthly life, have characteristic colors in ultraviolet and infrared light; these are largely confused by the Earth gases through which our present telescopes must look. A vantage point in space would also improve the performance of the telescope in observing small details which are now obscured by the shimmering of our air.

oratory at Stanford is focused on the detection of life by means of radio signals to be returned from an instrument packet landed on Mars. Given our exquisite ignorance of the



Joshua Lederberg is a man of many minds—all creative. His pioneering studies in the sexual recombination of bacterial cells won him the Nobel Prize in 1958. Lately he has branched into another field. For this one he has coined the name "euphenics." It foresees the control of human development through discoveries recently made or anticipated in molecular biology. Between the two he launched exobiology, and in it he finds a common strand for all of his work-and, in fact, for all of biology and medicinebecause the discovery of life on other planets would provide a fresh, unique approach to contemporary theory on the origin of all life.

Born in Montelair, New Jersey, in 1925,

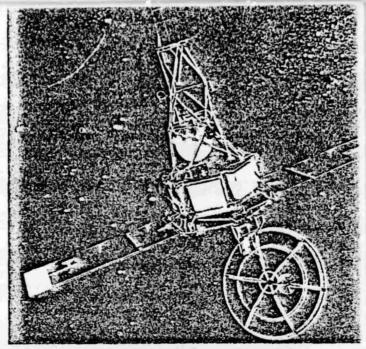
the son of a rabbi, Joshua Lederberg graduated from Columbia at 19. After two years as a medical student, he shifted to biology and obtained his Ph.D. a year later at Yale under the future Nobelist, Edward Lawrie Tatum. He and another Tatum assistant, Esther M. Zimmer, were married in 1946 and they continue as research partners. He is a stimulating conversationalist, interested in music, books, and his garden.

Dr. Lederberg taught 12 years at Wisconsin before coming to the Stanford School of Medicine in 1959. He is head of the Department of Genetics and director of the Lt. Joseph P. Kennedy, Jr. Laboratories for Molecular Medicine. He is a member of the National Academy. territory, how do we know what to look for and what tests and questions to build into our apparatus? The answer is that we don't, really; but this uncertainty provokes humility, not paralysis. In the first place, with the weight limitations in rocket payloads leaving us little room to mount a trap for bear, we shall content ourselves with examining a pinch of dust for microorganisms. Presumably we shall find them, if there is any life at all on Mars. At least, microbes are found everywhere on Earth—truly in all the air, waters, and soil.

What information shall we seek? We want to know if life on Mars has evolved in the same fashion as our own, how fast has it evolved, at what point might it have diverged from our pattern. We can predict with almost absolute certainty that Mars has achieved the chemogenic stage. Simple laws of physics and chemistry tell us that the presence of certain aggregates of atoms indicate there must be carbon on Mars. This is an a priori statement we can make without benefit of spectroscopic observation. We cannot know if the biogenic stage has been reached. As a matter of fact, we don't have a clear enough picture of terrestrial evolution to say that the earth moved from chemogeny to biogeny two billion years ago, or a half billion years earlier or later. And, of course, we cannot be sure that Martian life has not moved into cognogeny. Again, it is very difficult to predict how long it would take for a biogenic system to mature its information-handling capabilities into a cognogenic existence. We cannot discount this point; we have to include in our investigations not only inquiries as to whether or not intelligent life exists on Mars, but also whether or not it has been and gone.

Within this framework we can pose a series of rather explicit questions. We have converted a bewildering multidimensional state of possibilities into a reasonably linear array along which we can step up or down to find reasonable probabilities.

In our laboratory we are placing special emphasis on the detection of nucleic acids and proteins—the very complex molecules which are present in all forms of terrestrial life. We believe that the most important aspect of the study of extraterrestrial life is the possibility of learning in how many different ways life can evolve. Whether or not forms of life could be based on



The Mariner II gathered valuable data in a Venus fly-by mission in December 1962. (California Institute of Technology-Jet Propulsion Laboratory photograph)

substances other than nucleic acids or proteins or on related analogues of these substances is one of the most fundamental challenges to biological theory.

would only add, in conclusion, that it would be difficult to point to any practical fruits of our research—the sooner or later return of dollars and cents or gadgetry—although I think there will be some. As science becomes technically more complex and expensive, it must depend increasingly on the generosity of society. It would be tragic for both if obvious yield dominated the scientific quest; this would stultify science and would provide answers only to the problems that are already half-solved for our knowing how to ask the questions.

Expeditions beyond the earth may be among the most costly experiments so far undertaken, but they should warrant their cost as one of the very aims of the human adventure. Dante wrote of how Ulysses exhorted his companions to join his heroic journey on the great ocean: "Remember the seeds of your being: You were not made to live like beasts but to seek the fulfillment of virtue and understanding." This spirit of the first explorer of the western tradition moves us today.

This unforgettable passage (Inferno, 26)—"Considerate la vostra semenza: Fatti non foste a viver come bruti ma per seguir virtute e conoscenza"—is not well rendered in English.

PAYLOAD to



by ELLIOTT LEVINTHAL

or since Darwin—and before him Copernicus—has science had the opportunity for so great an impact on man's understanding of man." This is the evaluation of the search for extraterrestrial life made by the National Aeronautics and Space Administration in its recent A Review of Space Research. It is a position which has been taken as well by many scientists in their studies of the nation's space efforts.

This interest has given exobiology a high priority on planetary missions. The requirements of these missions have greatly stimulated the interaction of the engineering and biological sciences, yielding dividends to terrestrial biology and medicine. Such interactions are part of the NASA-supported program of the Genetics Department Instrumentation Research Laboratory in the School of Medicine, in addition to the instrumentation specifically for Martian exploration.

The instrument we are designing for the earliest missions must collect and analyze a few grams of Martian soil or dust for signs of microbial life, the specific identifying properties of which are, of course, presently unknown.

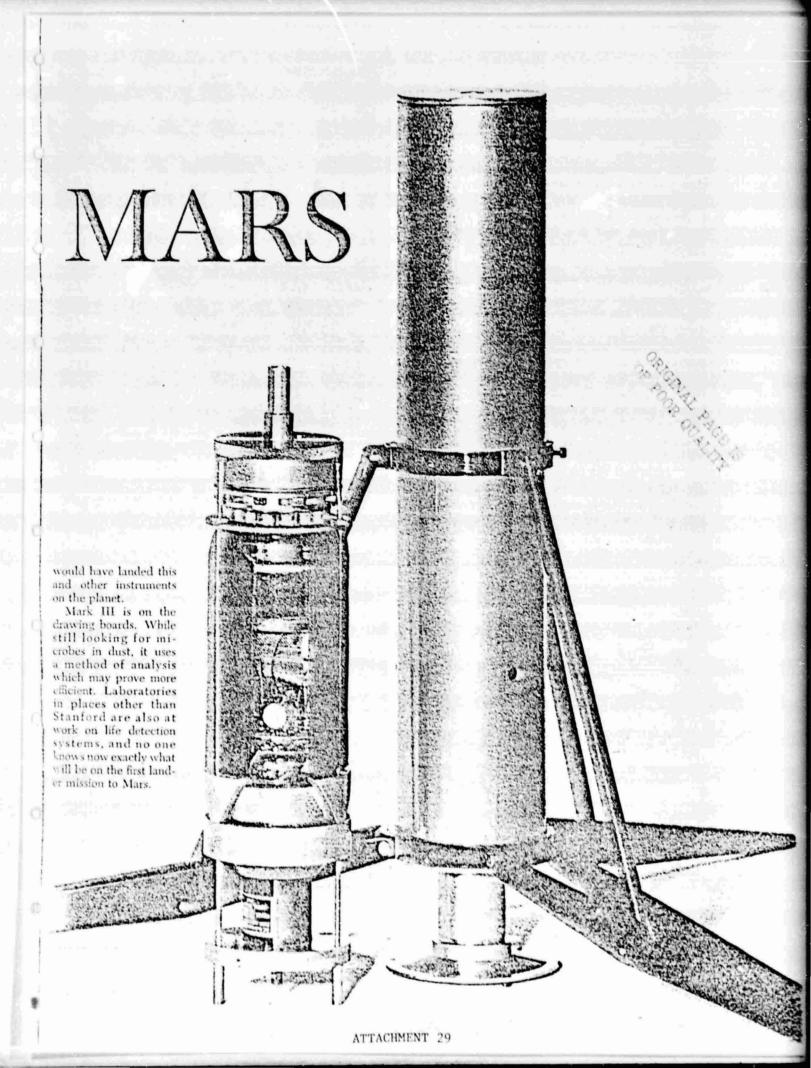
The results of the analysis must be in such form that they can be radioed, with very little power, 50 million miles back to Earth.

The instrument must be small, weighing but a few pounds, yet be rugged enough to withstand the impact of landing through a thin atmosphere, in high-velocity winds, on an unknown surface.

It must withstand sterilization—preferably by heating to 135° C. for 24 hours—and function reliably after lying dormant for six months of space flight.

And we must keep in mind constantly that the constraints

THE MARK II MULTIVATOR is an instrument for chemically analyzing a puff of Martian dust to see if it harbors microbial life. The package is just under 10 inches long and 2% inches in diameter. Its present weight of 30 ounces could be pared to meet flight conditions, but, actually, this little Multivator will never fiy. It is a bench model, a feasibility study to test the functio ing of its innards. On a flyable model, for instance, the folding legs would be equipped with an actuating device to bring the Multivator upright on the surface of Mars. And in the real thing an umbilical cord would connect to a radio trapmitter in the space capsule which



of the first mission to Mars are not yet known. Our designs must be readily adaptable to changes of many kinds before the payload is finally planned.

The first instrument—we call it a Multivator—being developed at Stanford does not yet meet all of these criteria, but we have gone far enough to believe that it can meet them all. There is a good chance that if a Mariner lands the first capsule on Mars in 1967 a Multivator will be aboard.

Missions to Mars must be carried out when that planet and Earth, in their orbits, are closest together. This situation occurs approximately every 780 days, and the experimental opportunities presently being discussed under the NASA programs are the six oppositions starting with March 1965 and ending December 1975. The first two are identified as Mariner missions and the last four as Voyager missions. They encompass possible instrument weights ranging from pounds to hundreds of pounds and radio links with information-carrying capacities varying from a fraction of a bit/sec to thousands of bits/ sec (at best a very restricted capacity, since standard telephone channels require thousands of bits/sec and television channels millions of bits/sec).

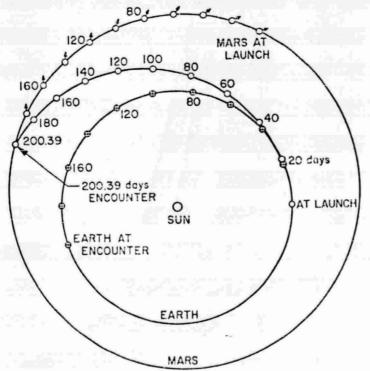


The real experimental heart of the Multivator, the part which is being intensively researched at Stanford, is the wheel of 15 reaction chambers and the connected test assembly which rest on top of the large photomultiplier tube.

We would like to use the knowledge acquired in one mission to determine the experiments in the next, but, since about two years separate oppositions, and the travel time is about six months, only 18 months remain to change plans. In the time scale of space missions this is very short. That is why we must look at the six missions in ten years as one large experiment and design a series of instruments and components which can be used as modules in different combinations and permutations based on the latest information. Modular design may cause some sacrifice on one particular mission, but we must accept this in order to have the inherent flexibility required by today's space science.

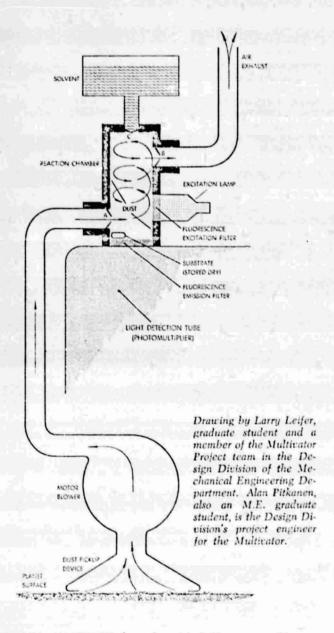
HE MULTIVATOR'S mission (and, of course, it would be only one of many instruments in the payload) is to discover the history and present stage of Martian evolutionary development. We are particularly interested to

3



A typical Earth-to-Mars spacecraft transfer orbit.

-Chart by Jet Propulsion Laboratory



THIS FLOW CHART shows how Mark II operates. Dust or soil is picked up from the surface of the planet by a device that resembles a little vacuum cleaner. It is filtered to retain only small particles and blown as an aerosol into the reaction chamber. The dust is extracted by a sticky coating on the chamber walls and the air is exhausted through a port. The air inlet and outlet are then sealed by valves (A and B). The solvent chamber valve (C) then opens and the solvent, probably water, is injected into the reaction chamber and the chemically reactive materials (called substrates), which were pre-stored dry in the chamber, are dissolved. The mixture is subjected to light from an excitation lamp. If the microorganisms are present in the dust, the light changes as a result of either fluorescence or absorption due to chemical changes, or of light scattering due to an increasing number of microorganisms. The change in light is detected by the photomultiplier tube. This information is reduced to digital form and transmitted back to Earth. Mark II has fifteen of these reaction chambers, arranged in a circle. One photomultiplier reading per chamber every 15 minutes is planned. Three of the fifteen chambers are blank-that is, they do not have inlet passages delivering Martian dust. These are designed for control tests to check the condition of the solvent and reactive materials after they have been subjected to the long space voyage and Martian environment.

know if life on Mars has experienced biogenic or cognogenic stages and if there have been any divergences from terrestrial experience. Even a sterile Mars, if it yielded historic traces of unsuccessful trials at life, would be extremely important in the study of life and its origins.

It is useful to distinguish between search and analysis. The earliest phase of the experiment looks primarily for signs of life. Having found such indicators, we use the knowledge acquired to ask more and more penetrating questions about its nature. This search-analysis dichotomy is sometimes obscured by a desire to combine the two steps into one experiment. This demands an experiment that is at the same time completely general and yet sufficiently rich in analytical details to give an unambiguously interpretable answer. The combined goal is sought not only because the prize of definitive proof of the existence of life elsewhere is so great, but also because of the possibility that, despite sterilization precautions, our capsule may contaminate the surface it lands on. This latter concern puts a high premium on the first-landed capsule giving an unequivocal answer. It also supports the conclusion that the first capsule should not be landed until fly-by missions have narrowed the matrix of possible chemical and biological properties that must be analyzed.

One of the assays we are considering for the first Multivator mission is aimed at detecting the enzyme phosphatase. We chose phosphatase because it is widespread-possibly ubiquitousamong terrestrial organisms; it catalyzes a wide range of reactions so that our choice of a substrate (the chemically reactive material to be stored in the Multivator's reaction chamber) is less risky; it is involved with the unique role of phosphorus in metabolism and energy transfer, which may very well be a universal characteristic of carbon-based aqueous living systems; and it can be detected with relatively high sensitivity. To detect this enzyme we use a substrate which does not fluoresce when excited by the light source. The phosphatase enzyme, if present in the dust sample, would interact very rapidly with this substrate. The products of this biochemical reaction do fluoresce; that is, they scatter light at a color different from that with which they have been excited. This fluorescent light is detected by the photomultiplier.

We have set as a goal the ability to measure



ELLIOTT LEVINTHAL—Program Director of Exobiology and Instrumentation Research Laboratory, Department of Genetics; took B.A. at Columbia, 1942, and M.S. at MIT, 1943, then worked for Sperry Gyroscope; received Ph.D. at Stanford in nuclear physics, 1949; was research director for Varian Associates; formed his own electronics company in 1953; informal discussions with Dr. Lederberg led to his joining the Genetics Department in 1961, broadening his field from electronics and physics into chemistry and biology.

PETER Z. BULKELEY—Assistant Professor of Mechanical Engineering and Acting Director of Design Division; a third generation Stanford man who took degrees at Bowdein and MIT before returning to the Farm to earn his Ph.D. in Engineering Mechanics in 1962; specialist in analytical design of machine elements.



EXOBIOLOGY AT STANFORD

WITHIN A FEW WEEKS after he became head of the Stanford Genetics Department in 1959, Joshua Lederberg established a research program in the search for extraterrestrial life. In January 1961 he delivered a landmark paper at the First International Space Science Symposium in Nice, France, bringing into general usage his name for the field—"exobiology." About a year ago the Genetics Department and the Mechanical Engineering Department teamed up when the latter's Design Division, under the leadership of the late John E. Arnold, undertook a hardware design program for the Mark II Multivator and which will be directed also toward the Mark III, the scanner-type instrument. Professor Arnold, who

in five years had brought the Design Division to a significant place in the School of Engineering, died of a heart attack in Italy last September. Peter Z. Bulkeley, already involved in miniaturization aspects of the Multivator project, became acting head of the Division. All of the work is supported by NASA—in the Genetics Department directly and in the Design Division through Jet Propulsion Laboratory in Pasadena. The instrumentation Research Laboratory was organized as part of the Genetics Department to carry out exobiology research. The laboratory is also applying the talents, facilities, and research of its space program to other areas in biology, some of which are of interdepartmental interest.



JOHN M. LESLIE—Lecturer in Design and head of Multivator Project in Design Division, Department of Mechanical Engineering; UC-Berkeley bachelor in electrical engineering, 1949; joined Ampex Corp. in 1950; long-felt interest in teaching led him to resign in 1962 as Ampex vice-president and general manager of Ampex Military Products Co. to come to Stanford for master's degree in Mechanical Engineering; now studying for Engineer degree, teaching, and engaged in design research.

L. LEE HUNDLEY-Electrical Engineer,
Exobiology and Instrumentation Research
Laboratory, Department of Genetics; received
bachelor's degree in electrical engineering
at Southern Methodist University, 1960; took
graduate study and worked in medical
instrumentation at Southwestern Medical
Center of University of Texas, Dallas; joined
Genetics Department in 1961.



incidences as low as 100 to 1,000 bacteria in samples of 1 to 10 milligrams of Martian soil. We have not yet reached this goal. The limitation does not seem to be the sensitivity of the assays themselves, but rather the chemical "noise" introduced when nonbiological changes of the substrate give false signals of the same kind we are trying to observe. A new technique is being tested. The soil particles are more or less evenly distributed over the surface of a substance, such as a gel, which contains the substrate. A spot scanner will then explore the surface for concentrations of fluorescent reaction products in the vicinity of microbial colonies, caused by the interaction of their enzymes with the substrate. The effective local concentration of the biological reaction products helps us to distinguish their signal from other chemical changes that occur throughout the whole substrate.

But Multivator's design philosophy is not tied to a particular assay, and scientists in laboratories other than our own could design experiments for it to carry out. This diversification is important because there can

never be a single, definitive detection experiment which will cover all possible manifestations of life. Our laboratory looks forward to these cooperative possibilities and intends to explore them.

For later missions, we can contemplate the possibility of actually observing Martian microbial life by means of a microscope which would transmit a video signal to receiving screens on Earth. Such a system, as well as methods of interpreting the visual data received, is being developed in our laboratory on a long-range basis; because of weight, it could not be used at least until the Voyager missions. And still further ahead, probably not until succeeding decades, Earth-developed vehicles may be able to penetrate to the major planets, especially Jupiter. These will present new opportunities for biological investigation. And the time will surely come when man will reach out to planetary systems beyond our own; in these may be found the first evidence of an intelligent form of life.

But for now we shall concentrate on our immediate problem—the development of instruments for detecting microbial life on Mars, our next-door neighbor in space.

Quotes

"About three-fourths of the total health expenditures in America come from private sources. Currently well over \$25 billion-or about 5 per cent of our gross national product-is spent on health. . . . This share is well above the 4 to 4.5 per cent of GNP spent on health in Great Britain, a country with governmental provision of health care for all. . . . The income of the average person in this country is now high enough so he can buy his own health insurance policies, his own retirement policies, not to mention his own cultural and recreational activities. This way he can obtain the combination of goods and services he desires most. According to the generally accepted tenets of economic theory, this should maximize his personal satisfaction."

-W. GLENN CAMPBELL Director, Hoover Institution "All countries that make the effort can produce all the food they need if they use the overabundant supplies of insecticides and pesticides, of fertilizers, and of cheap motor fuels for pumping irrigation water. . . . The North American continent was overpopulated when Christopher Columbus discovered it, because the Indians had only the skills to carve out a scanty living for their small population. Today, with 180 million people in the United States, we are seriously underpopulated by any standards we can reasonably apply. This country will not be overpopulated with 350 million or many more people and will have a much higher level of living."

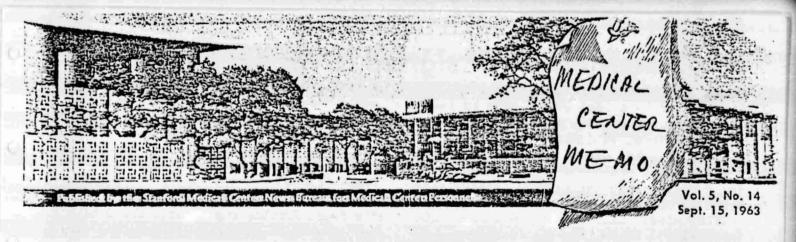
-Kabi, Brandt Director, Food Research Institute

"I like to believe that our nation would have made the effort, endured the trials and suffering that have come with the reassertion of the rights of the Negro in recent years, even if there had been no United Nations. However, I am also convinced that our government and our people have in fact been influenced by recognition that the position of the United States as a leader in the United Nations was prejudiced, seriously jeopardized, by the fact that almost 100 years after the Emancipation Proclamation, hundreds of thousands of our citizens had not been accorded the rights that were intended to come with their freedom from slavery."

—CANL B. SPAETH

-Cabl B. Spaeth Chairman, Committee on International Studies

"So long as we continue to take in students of the caliber of those now attending Stanford, I think we need have no fear that we shall fail in our task of equipping them intellectually, but I believe that we have a considerable task on our hands in trying to deal with their moral and spiritual education—although their receptivity for spiritual education is being exhibited to an astonishing degree at the present time.



Space Exploration Device Developed at the Center

Leading Hearing Defect Specialist To Join Faculty at Medical School

One of the country's leading specialists in hearing and hearing defects, Dr. Earl D. Schubert, will join the Medical School faculty next July. He will direct a new post-doctoral training program in audiology which will be established by the Division of Speech Pathology and Audiology next summer. He will have the rank of professor.

Only a few such programs exist in the U.S. It is being supported with a \$37,545 grant from the National Institute of Neurological Deseases and Blindness. The NINDB grant will be used to pay for salaries, postdoctoral fellowships and research.

Dr. Schubert has a wide knowledge of all aspects of audiology. He is particularly interested in psychoacoustics (why we hear sounds the way we do) and the neurology and physiology of hearing. Although Dr. Schubert has extensive clinical experience, his main interest is research and he has had many articles published.

Since 1960, Dr. Schubert has been professor of audiology at Indiana University. From 1955 to 1960 he was professor of audiology and associate in medicine at Western Reserve University and coordinator of research at the Cleveland Hearing and Speech Center.

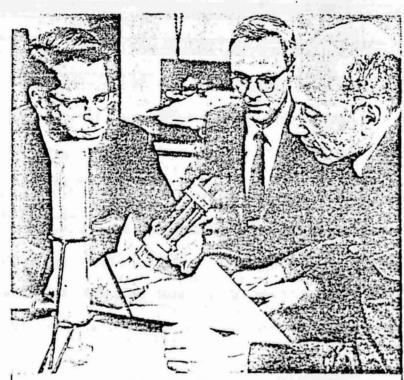
Dr. Schubert came to Stanford in the summer of 1962 as a visiting professor of speech pathology and audiology. He has also taught at the State University of Iowa and the University of Michigan.

Dr. Schubert received his B.S. from Manchester College in 1938 and his M.S. and Ph.D. from the State University of Iowa in 1942 and 1948.

A small instrument weighing just over two pounds and capable of a wide range of biological experiments has been designed at the Medical Center to investigate the possibility that life might exist on Mars.

The device, known as a multivator, is being considered for the first capsule mission planned for a landing on Mars.

It was developed in the Department of Genetics by Dr. Joshua Lederberg, head of the department; Dr. Elliott Levinthal, a research associate; and Lee Hundley, an electrical engineer. They were assisted by members of the Stanford Mechanical Engineering Department and the Jet Propulsion Laboratories in Pasadena. Physically, the instrument package is about the size of a (Continued on Page 3)



A MODEL OF THE MULTIVATOR is examined by Dr. Elliott Levinthal, Lee Hundley and Dr. Joshua Lederberg of the Department of Genetics.

Small Instrument Designed to Detect Life on Mars

(Continued from Page 1)
milk carton. It is just under 10 inches
long and about 2 3/4 inches in diameter.
It weighs about 30 ounces at this stage of
development, but may be miniaturized later.

Its purpose is to analyze a dust sample for microscopic organisms, since these would be found in every pinch of dust or puff of wind. Dust from the planet's surface will be blown into the reaction chambers within the multivator.

The dust will collect on a sticky coating on the chamber walls. A solvent such as water will be injected into the chambers where chemically reactive materials will already have been stored.

There are 15 chambers, three of which are blanks. The 12 working chambers will contain the reactive materials which will amplify certain steps in the metabolism, or chemical activity associated with life processes, of the microbes. Thus the presence of relatively few organisms can be detected. The blank chambers will serve as checks on the reaction to insure the reliability of the tests. Information as to the progress of any reaction within each chamber can be telemetered back to earth.

The multivator could also measure growth of bacteria and be adapted to use radio-active tagging techniques. Solvents other than water, different solvents in each chamber, and measurements of conductivity, acidity and the like, could be worked into the design.

In outlining the problems of detecting signs of life elsewhere than on earth, Dr. Lederberg said that one could look for any one of the three stages in the evolution of life on Mars:

- 1. The development of complex organic (carbon) compounds which are typically associated with life but do not necessarily indicate that life exists.
- 2. The development of more complicated compounds which are reproduced and which contain genetic information that specifies what organisms will be like. Errors in this reproduction, together with natural selection, bring about evolution and the great variety of earthly life.
- 3. The evolution of mechanisms for thought, perception, and the accumulation of knowledge associated with intelligent communication.

The multivator has been designed to examine the possibility that the second stage has occurred. The presence of organic ma-

terials would indicate biological processes could have developed, but would not provide a precise indication that life existed.

Current experiments proposed for the instrument are based on the assumption that life on Mars would depend upon the same basic carbon chemistry that supports life on our planet. Should this prove not to be the case, further experiments would have to be designed to test for alternative forms of life.

In attempting to analyze Martian soil for traces of life, the scientists hope eventually to be able to tell whether Mars took the same general path as the earth in the chemical development of life, and how it diverged at any of the stages of evolution.

The multivator was developed under a grant from the National Aeronautics and Space Administration to the Instrumentation Research Laboratory of the Department of Genetics.

Menninger Information Specialist Joins Medical Center News Bureau

New associate information officer and director of the Stanford Medical Center News Bureau is Spyros Andreopoulos, formerly of The Menninger Foundation.

He will assist the information officer, Samuel Moffat, with news stories and publi-

cations about the Center. Initially, he concentrate nontechnical material, with emphasis on the Hospital's information program. He will supervise the Medical Center Memo, which will be written by Pat Black.

For the past four years Mr. Andreopoulos has been assistant



SPYROS ANDREOPOULOS

director of information services at The Menninger Foundation in Topeka, Kansas, and editor of the Menninger Quarterly.

Prior to that he was a journalist in Wichita, an information specialist in Greece, and a public information officer in Japan. He obtained his B.A. from the University of Wichita.

SIGNS OF LIFE

A Survey of the Detection Problem In Exobiology

by

Joshua Lederberg
Department of Genetics, Stanford University Medical School

Introduction

The maturation of exobiology calls for increasing attention to the systematic statement of its theoretical basis and its operational methods. Very little science is totally irrelevant to it and the policy-maker faces the danger of utter confusion in reacting to a flood of isolated proposals for the development of spaceflight experiments. The stakes of such a large enterprise even demand investment in new methodologies requiring further emphasis on the choice of valid goals, rather than available means which are tolerable bounds to more individualized efforts in other fields. A system should help dispel this confusion and rationalize the division of labor, the only means of reducing a complex problem to manageable parts.

As a target Mars takes first place in our present thinking, yet our premissed information is only (1) terrestrial biochemistry, (2) the inferences from labelling Mars as a "terrestrial planet", and (5) a very small body of definite observational data. Thus the choice of our first experiments must take account of a wide range of theoretical possibilities. Our speculation will be narrowed and therefore simplified by any tangible information about Mars, even much that may seem to fall outside the domain of biology. Such information is increasingly valuable if (as most global studies overlook) it encompasses the variability of the planet's features in space and time.

. Evolutionary Stages

Fundamental to all biological theory, eso- or exo-, is the evolutionary principle. As is now commonplace, we name the following stages in the Earth's history:

A. Chemogeny (Organic Chemistry)

The production of complex organic compounds by a variety of <u>non-replicative</u> mechanisms - the primitive cosmic aggregation, photochemistry of insolated atmospheres, thermal, inorganic-catalyzed, and spontaneous reactions of previously formed reagents.

B. Biogeny (Biology)

The replication of a specifically ordered polymer, e.g., DNA, which specifies the sequence of its own replicates, and of RNA and proteins, from which cells and organisms are fashioned. Random experiments of error in

replication, and natural selection of their developmental consequences, result in the ranoply of terrestrial life.

C. Cognogeny (History)

The evolution of the mechanisms of perception, computation and symbolic expression whereby interpersonal communication can occur and tradition can accumulate, i.e. culture.

Mars must be supposed to have had an earlier history similar to Earth. Our question is then, how far has its chemogeny gone; how like and how unlike the Earth's; has its evolution passed through the biogenic (ordered macromolecular) stage? Then through the cognogenic?

In evaluating a complex set of possibilities it is helpful to find a classifying parameter that can be scanned systematically, if sometimes only implicitly, to generate a probability space. In this case, the evolutionary principle furnishes the parameter: chemical complexity. Of all chemical possibilities, terrestrial life comprises a set of choices: to what stage of complexity has Martial evolution progressed, and at what levels has it diverged from the terrestrial?

For other planets, for example Jupiter, the hypothesis of ultimate divergence is more plausible than for Mars. If only to evade perplexity how to deal with a totally unspecified situation, we state, but pass over the possibility of a non-aqueous or non-carbonaceous system, that is we postulate that Martian life is predicated on chemical linkages, predominantly -C-C-, -C-O-, -C-N-, and -O-P-, that are barely stable in aqueous medium. We leave to hypothesis the extent to which the constructions from these radicals emulate terrestrial blochemistry at each level of complexity.

Chemogeny generates a vast mix of products through the level of random macromolecules. Whether or not it had progressed to biogeny Mars must have nurtured
such chemistry. A negative assay for organic materials would preclude biogeny,
but would properly be blamed on deficiencies in the particular sample. The
positive assay, if it told something of the concentration and composition of
organic molecules, would add to our understanding of Mars' development, and would
contribute to our judgment of the life-detection problem. But it would not answer
it. On the other hand, once life has appeared on a planet, it would dominate
its organic chemistry - most carbon compounds would be witnesses of biogenic
(or cognogenic) specificity. The description of organic molecules has at least
the second-most priority in exobiology.

A scan of chemical entities points up many conceivable data hard to reconcile with chemogeny, and thus imply biogeny or cognogeny. The simplest example: Suppose a specimen of water contained pure H (to the exclusion of deuterium).

Such samples do exist on Earth, but the probability that a randomly chosen sample will have such a striking artifact is small, smaller still if we prefer not to take account of the products of cognogeny.

The chemical scan

To publish a complete scan through chemical complexity might be as witless as it is pretentious for any one person to attempt. However, segments of such a scan

are challenges to the imagination of the specialist, as the scrutiny of the first element, hydrogen, already hopefully suggests. Just what discrepancies in the H²/H¹ ratio in a Mars sample would be beyond simple (chemogenic) explanation? How would one interpret such a finding from a terrestrial foray? What are the simplest instrumental means of making such a measurement? What are the ranges of biogenic, of cognogenic systems that might be expected to generate discrepant samples? What is the probability of detecting such a sample?

Intuitively we judge this probability very low, but this is a tentative judgment mainly based on terrestrial experience (the market price of pure protium!). We cannot formally prove the impossibility of natural fractionation processes as an alternative to biogeny, without specifying the local circumstances.

The entropy argument

More important than the particular nuggets that we might hope to find by a systematic scan are the generalizations that impatience or weariness might impel.

Given the evolutionary continuity of life and our understanding of the organism as a chemical machine, there can be no absolutely distinctive signature of life. Some conjunctions - like a planetary depot of protium - would be so unaccountable to our present model of chemical behavior that we would feel obliged to postulate the operation of a goal-directed system (biogeny or cognogeny) rather than accept the improbability of such a conjunction by chance. This choice plainly depends on our freedom of choice of models. For example, our present knowledge of chemogeny permits a wide latitude of hypotheses as to the range of molecular species that atmospheric photochemistry might generate. Further developments in our knowledge of chemogeny or of the available chemical and physical resources of Mars might confer useful constraints on the date that might be "explained away" as chemogeny, and thus cannot make a critical contribution to our search.

From terrestrial experience we judge that the occurrence of any of a number of compounds in high purity is a sign of life. Such deposits at a microscopic level are even more likely to signify cognogeny - a smelter, a chemical laboratory, a communications cable, than biogeny - organic structure usually being built of microscopically defined components. Pockets of entropy are not unique to life, however, and only the details of experience or confident use of available theory can decide whether the eddy has a chemical-kinetic explanation or a bio- or cognogenetic one. Lacking our experience, a Martian visitor might credit the association of diamonds to some mysterious biogenetic function, inhibited by Y chromosomes; if he were cleverer, to the General Electric Company. He would need very special knowledge of the Earth to predict they would be found in the ground.

The entropy argument can be generalized further to improbability values in an open system. Thus the accumulation of kinetically unstable materials (in the context of local chemical and physical conditions) would also call for a special explanation. For example, an accumulation of photosensitive pigments (witness terrestrial chlorophyll) requires special attention to the magnitude of plausible synthetic processes (atmochemical vs. biogenic) by which their steady-state concentration could be maintained. Analogous reasoning would apply to compounds that are thermolabile in relation to the ambient temperature, or chemically unstable species that should reach equilibrium with oxidants and other reagents.

Optical activity

The discrimination of optical isomers is the most promising entropy pocket turned up by the chemical scan. In fact, for any carbonaceous (more generally, tetravalent) system, substantial net optical asymmetry is virtually equivalent to biogeny. Our fundamental definition of biogeny is the well-ordered macromolecule (we have still to discuss a direct approach to its detection). tetravalent carbon is incorporated into macromolecular structure, each carbon stands a reasonable risk of being asymmetric, of having a distinctive substituent on each of its four valences. Such an atom is subject to stereo-(optical) isomerism, and its orientation, D- or L-, must be specified if the macromolecule is to be ordered, more concretely, if it is to have a well-defined threedimensional shape. Conversely, biogenetic macromolecules, having ordered asymmetric centers, have the necessary information to discriminate among the isomers of monomeric substrates. It is less obvious why only L-amino acids are used for terrestrial proteins. The Intercalation of a D- amino acid would be a new element of versatility. We may know better when the rules of polypeptide conformation are better known. Or the enswer may be in the details of evolution of amino acid anabolism and problems of discrimination of analogs. Howbelt, optical isomers will not occur at exactly the equivalent concentrations in any biogenic system. The theory has ample support from terrestrial experience. The ratio of D-glucose to L-glucose on the Earth must be better than 1015:1. In addition to the theoretical generality and historical tradition of the Pastorian principle, the criterion can be applied to any organic molecules or an aggregate of them. Paradoxically, optical activity is instrumentally a weak measure and its historical preeminence might do ill service by obscuring the basic criterion: a statistical preference beyond chance or weak chemical effects, among asymmetry isomers. The matter is discussed further under Instrumentation.

Signals

The main tack of search for cognogeny is the scrutiny of radiation emanating from the planet rather than from its chemical composition. But similar considerations apply to the detection of intelligent signals: If the coherence or monochromaticity or modulation pattern or other regularity of the signal defeats our efforts to attribute it to some natural mechanism, we have only the alternative of purposive behavior. The spontaneous emission of a prime number series; even more, an intelligent reply in dialogue are extreme examples, but still fall within this criterion. (A coding theorem reminds us, however, that the most efficient communication of information is by definition, indistinguishable from noise to the unbriefed eavesdropper.)

Flyby or orbiter missions may be more efficient at detecting the radiation signals and large-scale topographic modifications expected of cognogeny. Landers may well be planted on Mars before there has been extensive surveillance to settle this question, and simple assays for "animal", especially cognitive life should not be overlooked. These might range from ideographic descriptions or the origin and scientific purposes of the experiment, and instructions how to modulate the telemetry; "Martians, please press the black button, do not press the red one", to night-lights, microphones, and acceleration detectors. The latter in particular should be able to discriminate between some classes of physical events for example, sudden impacts - from those characteristic of manipulation by an animal. With the suggestion that this problem has not received the attention

it deserves, we leave its further consideration to other occasions.

Macromolecules

Macromolecules of well-defined, information carrying order bound chemogeny from biogeny. The replication of such macromolecules (genes) and the possibility of random error (mutation) opens the door to natural selection and the evolution of more and more complex forms of life. The most direct challenge of exobiology is the assay of informational macromolecules. A random polynucleotide is not life; routes to its photochemical synthesis from simple gasses and inorganic phosphate are in sight. But we might find evidence of its replication, either by direct observation, or by finding a significant concentration of replicas of the same sequence. The sequence need not be the gene itself. Macromolecular sequencing is also manifest in gene products, RNA and proteins. It is important that the sequence imply ordering from a template which selects from an abundance of kinetically equivalent choices, not marely a pattern inherent in the chemistry of the monomer, as in crystallization.

The main methodological problems of contemporary molecular biology are exactly those which face this area of exobiological work. This challenge gives us the groundwork for exobiology and assures the full utilization of any instrumental advances. But it is a chastening note that biochemistry has - as of new - perhaps barely reached the point of affirmation that antibody gamma globulin answers these criteria in any detail. Indeed, some workers dispute that this protein is synthesized by the general rule of information transfer from DNA. That this question concerning an abundant and important molecule can still be in dispute at the present time warns us of our limitations in answering analogous questions concerning macromolecules on another planet.

How do we detect informational macromolecules?

- A. Compositional Analysis
 - 1. Demonstration of macromolecules in the sample.
 - Demonstration of composition and nonrandom ordering in such macromolecules.
 - The most evident approach to A, and the foundation of blochemistry, is the isolation of macromolecular species from the sample, and their purification before attempts at analysis. Practical methods are mostly empirical, far from general principles of wide application - various regimes of extraction and precipitation - which does not preclude their usefulness if the sample collection and processing equipment permit.

More rational techniques mainly rely on diffusional properties of large molecules, free diffusion, sedimentation, dialysis, molecular sleves, and electrophoresis, in principle also vapor phase diffusion (to remove monemers) - molecular distillation, gas chromatography, and mass spectrometry. Solution chromatographic methods may also rely on the coincidence of functional groups on one molecule, e.g., a polyelectrolyte.

Similar principles underly non-separative methods of detection which have not been extensively developed to date. Rotational relaxation times

can be measured by flow or electric birefringence, or the analogous polarization of fluoroescence. Polyfunctionality is tested by intermolecular interactions of adsorbed dyes (e.g. optical shifts in acridine orange on DNA) or the monomeric units with one another in special cases (hypochromicity of DNA, diagnosable upon heat-denaturation). More direct chemical tests for polyfunctionality also suggest themselves.

2. The previous methods, insofar as they lack perfect generality, may give some clue as to the composition of the macromolecule, as well as its molecular size. At the other extreme, we would seek the complete primary structure to emulate the recent tours-de-force of chemical technique. Reasonable inferences might be drawn from less complete evidence of structural individuality, hard to evaluate in advance: homogeneity in molecular weight or endgroup analysis, crystallinity, or sharp fractionation by any other procedure. A sharp X-ray diagram of a heteropolymer sample could imply its individuality long before it had yielded to full analysis.

Other partial measures of great utility include the scission of the polymer by specific reagents, especially enzymes, to give a pattern of characteristic fragments (the polypeptide "fingerprint").

B. Functional Analysis

The uses that biogeny has discovered for macromolecules furnish other avenues for their detection. These functions are all reducible to the stereospecificity of the polymer in complex formation. The chemical specificity of complex formation then becomes the argument for the structural individuality of the polymer.

Function	Complex with	
Auto-Replication	n incipient polymer (sa building monomer	mme species) and polymer
Hetero-Replicat	ion Incipient polymer (di building monomer	fferent species) and polyme
Morphogenesis (Fibers, Membra Vesicles)	Formed polymer, similares,	ar species
Enzyme	Substrate - catalytic Cofactors - to form h Analogues - complexes	c effect noloenzyme s Inactive, qua enzyme
Neutralizing (Inducible - an	Antigens ntibody)	of the designation of the
Transport (e.g. serum alb	Hormones, toxins	

In this list, the enzymatic functions are particularly promising in light of their specificity and amplifying capability. Many enzymes have turnover numbers of 10⁴ substrate molecules per second per enzyme molecule. If suitable precursors (nutrients) can be defined, enzyme sequences, e.g. respiration or photosynthesis, extend the versatility of this approach. Finally, replication, be it of molecules or cells, offers the largest amplification - a single bacterium could grow into tonnage masses in a few days, but might be the most exacting of the environment.

Metabolism

This is an extension of the concept of testing for enzymatic activity. Under certain conditions the test for a sequence of enzymatic reactions, the metabolic system, can exploit the improbability of its simulation by a non-biogenic process. On the other hand, the same condition also decreases the a priori probability that the entire sequence will be represented in an extraterrestrial species. This a priori probability will be greatest, of course, the simpler the level at which the metabolic reactions are tested: for example, the assimilation of elementary nutrients, e.g. C, N, O or P into organic molecules. At the next highest level of chemical complexity, molecules such as H₂O, CO₂, and O₂ and NH₃ are among the most pervasive metabolites of terrestrial life, and the choice among them for searching for evidence of their conversion into other compounds will depend mainly on instrumental considerations. In general, the more complex the metabolite being tested, the less assurance we would have that it was part of an extraterrestrial biogenic system.

Instrumentation

The classification of existing instruments, or those proposed for analytical purposes is a task as difficult as it is urgent. The real aim, a classification of possible instruments, requires a total knowledge of physics. However, if human limitation precludes perfection, some system may be better than none, and we can lay one out even if we cannot exhaustively analyze it. A proposed scan parameter is the energy level of the transition by which the molecule is recognized. Further parameters include whether photons are introduced or emitted, whether chemical reagents are employed, including auto-reactions, whether the displacement of state (including position) of the analysand or the probe is diagnostic, and for radiation probes, whether power, polarization, phase, wavelength, or flux vector of the probe is altered as an index of the analysis.

Empirically, radiation probes have limited selectivity, but may be of special value in conjunction with chemical reagents. Absorption (power loss) measurements have dominated instrumental analysis. But conventional methods rarely stabilize or measure input power better than 1:1000, with corresponding limitations to detectivity. For example, optical molar absorptivity rarely exceeds 105 so that 10-8 molar solutions (6 x 1012 molecules in a 1 cm3 cell) would give the lowest useful signal under the most favorable conditions. By contrast, fluorometric measurement (which can exploit shifts in wavelength, flux vector, polarization and phase) can easily measure 108 molecules and can probably be extended to 104 under favorable conditions. The delicacy of excitation methods (which could also include chemical, nucleonic and thermal excitation) stems from the measurement of a signal against a noise background rather than against the power fluctuations of the probe.

Optical activity is usually measured via power loss (attenuation of polarized light by a crossed analyzer): the molar rotations are relatively small, present detectivity being about 1015 molecules.

The most sensitive approaches to analysis are two-stage mechanisms: the <u>selective</u> <u>displacement</u> of the analysand, then a <u>sensitive detection</u>. In principle, such methods might detect a single molecule, as in mass spectrometry: m/e displacement followed by accelerated ion detection. The reasoning behind this recommendation can be illustrated by its application to optical activity (stereo-asymmetry) on a Mariner-type mission.

We assume capture of a dust sample of, say, 100 mg, containing at most 100 µg of organic matter, perhaps 1 µg (about 10 nanomoles) of a particular species. Direct measurement of optical activity of such a dilute sample is far beyond present technique. Typical of the analytical problem is a very small sample which, however, has a marked bias in the molecular proportions. It might contain, or be converted, into, say 100 molecules of D-lactic acid, plus only 104 of L-lactic. Diffusion techniques can be devised to separate such species, chromatography on an active adsorbant, diffusion through an active membrane - even more certainly after complexing with an optically active ligand (say R₁ R₂ R₅ Si-) giving chemically distinct diastereoisomers. These tactics transfer the problem to the much smaller dimensions of chemical identification.

A science of metrology, the orderly study of methods of measurement, remains to be developed. The preceding classifications, lacking such a thought out theoretical framework, are untidy, and thus lend less confidence as to their completeness than would be desired. (Reprinted from Nature, Vol. 207, No. 4992, pp. 9-13, July 3, 1965)

SIGNS OF LIFE

CRITERION-SYSTEM OF EXOBIOLOGY

By PROF. JOSHUA LEDERBERG

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THE imminence of interplanetary traffic calls for systematic criticism of the theoretical basis and operational methods of 'exobiology', the initial search for and continual investigation of the life it might encounter. Very little science is totally irrelevant to it, and the policy-maker must face a riot of potential approaches to space flight experiments. By every standard, this is an epochal enterprise: a unique event in the history of the solar system and of the human species. and the focus of an enormous dedication of cost and effort. It requires a new perspective in experimental policy. The broader interfaces of eso-(Earth's own) biology, by contrast, permit its fruitful growth within the context of methodologies and instruments that can lag behind broadly established needs and imaginative possibilities. A system for orderly appraisal of the problem would rationalize the partition of labour, our only means of managing a complex problem.

Mars is our prior target. Our premissed information is only: (1) terrestrial observation: esobiochemistry; (2) the implications of Mars being a 'terrestrial planet'; (3) a very small body of definite observational results. The choice of our first experiments must take account of a wide range of theoretical possibilities not yet narrowed by the experimental process. Over this broad reach, logical necessity rarely coincides with logical sufficiency. The most compelling inferences might stem from the least likely event. Our speculation will be narrowed and policy simplified by tangible information about any aspect of Mars, especially if it encompasses the variability of the planet's features in space and time.

Evolutionary Stages and the Definition of 'Life'

Fundamental to all biological theory, eso- or exo-, is the evolutionary principle. As is now commonplace, we recognize the following stages in the Earth's history:

(A) Chemogeny (organic chemistry). The production of complex organic compounds by a variety of non-replicative mechanisms—the primitive cosmic aggregation, photochemistry of isolated atmospheres, thermal and spontaneous reactions of inorganically catalysed, previously formed reagents.

(B) Biogeny (biology). The replication of a specifically ordered polymer, DNA being the terrestrial example, which specifies the sequence of its own replicas, and of the working materials, like RNA and proteins, from which cells and organisms are fashioned. Random experiments of error in replication, and natural selection of their developmental consequences result in the panoply of terrestrial life.

(C) Cognogeny (history). The evolution of the mechanisms of perception, computation, and symbolic expression and interpersonal communication, whereby tradition can

accumulate, culture unfold.

Mars must be supposed to have had an initial history similar to Earth. To ask whether Mars has life is to ask how far has its chemogeny gone; how like and how unlike the Earth's; has its evolution passed through the biogenic (ordered macromolecular) stage? Then through the cognogenie?

In evaluating a complex set of possibilities it is helpful to find classifying parameters which can be scanned systematically, if sometimes only implicitly, to generate a probability space. In this case, the evolutionary principle furnishes the parameter; chemical complexity.

The initial planetogeny and the consequent differences in physical and chemical environment determine the possible points of departure of the evolutionary processes. On these grounds, Jupiter must have special interest for comparative cosmochemistry; but it is still much less accessible to close investigation, and we have even less or a basis to predicate a homologous chemogeny there than we do for Mars. In so far as Mars does retain some environmental analogies to Earth we might at least predicate, for one branch of our analysis, that any Martian life is based on chemical linkages, predominantly C.C., C.O., C.N.— and O.P.—, which are barely stable in aqueous medium. We leave to hypothesis the extent to which the constructions from these and other radicals emulate terrestrial biochemistry at each level of complexity.

The cosmic abundance of these elements is relatively high, and there is every reason to believe that Mars is at least as richly endowed as Earth in them. If the initial budget of carbon has not, like that of the Earth's crust, been completely requisitioned by life, then what form

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will we find it in ?

Chemogeny generates a vast mixture of products through the level of random macromolecules. Mars must have nurtured such chemistry, whether or not it had progressed to biogeny. A negative assay for organic materials would preclude biology, but could we believe such a result? It would properly be blamed on deficiencies in the particular sample. The positive assay, if it told something of the concentration and composition of

organic molecules, would add to our understanding of Mars's development, and would contribute to our judgment of the life-detection problem. But it would not answer it. On the other hand, once life has appeared on a planet, it would dominate its organic chemistry—most carbon compounds would be witnesses of biogenic (or cognogenic) specificity. The cataloguing of organic molecules is a description of the consequences of evolution and must make up a large part of our effort.

The Chemical Scan

To promise an actual complete scan of hypotheses of molecular complexity would be pretentious and witless, notwithstanding that a computer can now be programmed to visualize all the possibilities. However, the fantasy of such a scan is a constructive exercise in evaluation of evidence for life. For each chemical species the imagination of the specialist might be challenged to ask:

(a) is there any information concerning the existence of this item relevant to scientific inference in exobiology;

(b) what are my prior expectations on the distribution of this species, with and without life; (c) what other data could contribute; (d) how would the observation be interpreted from a terrestrial foray; (e) what special methods are available or could be devised to detect the species?

We might nurture a hope of turning up a special treasure, a rare example of a molecule, which would reveal something about the evolution of the planet and help narrow our choices among the confusing array of possible targets. In practice this advantage does not materialize so easily, for the hope is false. Not that no chemical

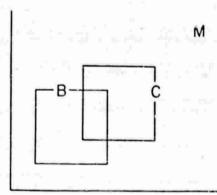


Fig. 1. Contingency space. The universe of possible observations I includes the overlapping domains B and C, the predictions of biogeny and chemogeny, respectively. The remainder is M, contradictions of these models, and possible consequences of engageny. Should this also be bounded.

ORIGINAL PAGE IS

species is potentially informative; paradoxically, every one is,

Consider hydrogen. In terms of the simple Venn diagram (Fig. 1), most expected observations would fall in the region ($B \cdot C$), that is, would be consistent with biogeny but not imply it. However, the sensible abserce of hydrogen from Mars' surface would fall in region ($-B \cdot C$), that is, virtually preclude life. But if we could produce no plausible physical model for the disappearance of hydrogen, we would have to reconsider the region ($I \cdot -C$), that is, to ask whether the anomaly implies a biogenic or cognogenic sequestration of the element. On the other hand, certain microscopic distributions of H are hard to reconcile with any chemogenic model, and point to the region ($B \cdot -C$), that is, an inference in favour of biogeny. This verges on morphology, but can still be formulated as molecular statistics.

On another tack, suppose a specimen consisted of pure protium, ¹H, to the exclusion of deuterium, ²H. The price of pure protium on the terrestrial market hints at the obstacles to a chemogenic model. Apart from cognogenic activity, if a biogenic system were exquisitely sensitive to deuterium-toxicity it might evolve a discrimination against it.

The arguments have been laboured, but are quite typical of those that discovery of any other species would arouse.

Entropy or Unlikelihood ?

Given the evolutionary continuity of life and our understanding of the organism as a chemical machine, there can be no absolutely distinctive signature of life. Some conjunctions-like a planetary depot of protiumwould be so unaccountable to our present model of chemical behaviour that we would feel obligated to postulate the operation of a goal-directed system (biogeny or cognogeny) rather than accept the improbability of such a conjunction by chance. This choice plainly depends on our freedom of choice of models. For example, our present knowledge of chemogeny permits a wide latitude of hypotheses as to the range of molecular species that atmospheric photochemistry might generate. Further developments in our knowledge of chemogeny or of the available chemical and physical resources of Mars might confer useful constraints on the data that might now be 'explained away' as chemogeny, and thus cannot yet make a crucial contribution to our search.

From terrestrial experience we judge that the occurrence of any of a number of compounds in high purity is a sign of life. Such deposits at a macroscopic level tend to signify cognogeny—a smelter, a chemical laboratory, a communications cable, rather than biogeny—organic

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structure usually being built of microscopically defined components. Negentropy is a necessary, but not sufficient, sign of life. However, it can help filter out the most promising situations. Then only the details of experience or confident use of available theory can decide whether the eddy has a chemical-kinetic explanation or a bio- or cogno-genic one. Lacking our experience, a Martian visitor might credit diamantane carbon to some mysterious biogenic function, inhibited by Y chromosomes; if he were eleverer, to the General Electric Co. He would need very special knowledge of the Earth to predict that diamonds would be found in the ground (and even mere to understand why men dig them up, only so that women will wear them).

Kinetic instability in the context of local chemical and physical conditions is another clue. For example, cover of photosensitive pigments (witness terrestrial chlorophyll) requires special attention to the magnitude of plausible synthetic processes, atmospheric-chemical versus biogenie, by which their steady-state concentration could be maintained. Analogous reasoning would apply to compounds which are thermolabile in relation to the ambient temperature, or chemically unstable species which should reach equilibrium with coexistent exident. Do we see a forest fire? Then we must think of the efficient system of photosynthesis which will restore the steady-state vegetation. Top-heavy structures, which high-altitude reconnaissance could perceive even without resolving single trees, houses, or bipeds, likewise tell of kinematic instability, and in turn, some process to reraise what must some time fall. But geophysics competes with biophysics, and we have to discriminate life from vulcanism and orogeny.

In sum, unlikelihood in terms of the chemogenic model gives weight to any finding as a datum for exobiology. It should be possible to quantitate chemogenic likelihood, essential if a datum is to be given a measured value in any decision-making programme. The resolution of the measurement need not be very high to make it still very useful in comparing dispurate approaches.

In more general terms, biota have a high density of internal information: the root of our conceptual distinction between matter and life is the rich story that life can tell about itself, a plot the details of which we can searcely deduce from our simple knowledge of the initial conditions. But there must be a plot, that is, the information must have some interesting pattern, or we would not distinguish a cell from the dislocations in a snowblake.

Optical Activity

Many molecular species can contribute in an important way to our appreciation of life. A priori, we have a very limited basis to predict which species will be most cogent. We should, of course, give high, but not exclusive, priority to terrestrial prototypes like amino-acids and nucleotides. Fortunately, there is a generic classification of compounds which is relatively independent of detail of structure, yet should pervade a biogenic chemistry.

This is optical activity.

The argument for logical necessity of net optical activity has nothing to do with optical rotation. It depends on the crucial role of the informational macromolecule in a definition of life. When tetravalent carbon is incorporated into macromolecular structure, each carbon stands a reasonable risk of being an asymmetric centre, of having a distinctive substituent on each of its four valences. Such an atom is subject to stereo-(optical) isomerism, and its orientation, D. or L., must be specified if the macromolecule is to be fully ordered, more concretely, if it is to have a well-defined three-dimensional shape. versely, biogenic macromolecules, having ordered asymmetric centres, have the necessary information to discriminate among the isomers of monomeric substrates. On Earth, where biogeny has dominated the statistics of organic molecules, we find that the ratio of D- to L-glucose residues is at least 1013:1.

Logical sufficiency can also be argued. Chemical enantiomerphs should be generated in equal proportions
except under the influence of a catalytic system which is
already asymmetrically organized. The global organization of a planet into one catalytic system of particular
orientation is a catastrophe of a magnitude unique to
biogeny. Spontaneous resolution might occur locally.
Hence this criterion has its greatest weight when applied
to a species which flows through the planetary circulation.
A paragon would be labile molecules condensed from the

atmosphere: optically active smeg.

Within the biogenic system, both enantiomorphs of a metabolite might be generated, but this would be in precisely equal amounts with no greater likelihood than any two species designated at random. Chemogeny and sterically ordered biogeny thus give sharply contrasted expectations on these statistics, and biogenic chemistry can hardly avoid becoming sterically ordered.

These lines of inference do not account for such parochialisms as the undeviating series of L-isomers of aminoacids in esobial proteins. For example, p-alanine would be at least as interesting as L-valine in expanding the homologues of glycine. But, for that matter, why is z-amino butyric acid passed over? We may know better when the rules of polypeptide conformation are better known; more likely, from the details of evelution of amino-acid anabolism and problems of discrimination among analogues. It is precisely at this level that the local biological theory fails, and thereby points to the crucial issues of a cosmic biology.

In view of the theoretical generality and historical tradition of the Pasteurian principle, it is paradoxical that the direct measurement of optical activity is weak by comparison to other instrumental approaches. However, the basic criterion is not optical rotation but molecular statistics. Enantiomorphs can be assayed with optically active reagents to give resolvable diastereo-isomers, and exploit the most sensitive methods known to chemistry.

Macromolecules

Informational macromolecules define the boundary of chemogeny and biogeny, of chemistry and life. Their description on another planet is the fundamental challenge of exobiology. Replication of macromolecules (genes) and the inevitability of random error (mutation) open the door to natural selection and the evolution of more and more complex forms of life. A random polynucleotide is not life; routes to its photochemico-synthesis from simple gases and inorganic phosphate are in sight. Can we deduce the replication of a polynucleotide by any means short of the most recent achievements of direct observation and in vitro enzymology? Historically, we could deduce the informationality of macromolecules just from compositional data. When the same sequence occurs in many molecules-as in a sample of crystalline haemoglobin-we have to invoke an informational process to programme and implement the synthesis of the protein. In fact, only recently and rarely could we gain complete specifications of an actual sequence. This is usually inferred from fragmentary analyses of a fraction found to be monodisperse on a few measures and then assumed to be sequentially homogeneous.

The sequence need not be the gene itself. Macromolecular sequencing is also manifest in gene products,
RNA and proteins. It is important that the sequence
imply ordering from a template which selects from an
abundance of kinetically equivalent choices, not merely a
pattern inherent in the chemistry of the monomer, as in

crystallization.

Molecular esobiology faces the same methodological problems. This challenge gives us the groundwork for exobiology and assures that any instrumental advances will have redoubled utility. But it is a chastening note that biochemistry has barely reached the point of affirmation that antibody γ-globulin has an informational sequence or is specified by a polynucleotide. That this abundant and medically important molecule can still be so controversial must evoke some humility in our postulations and experimental efforts concerning macromolecules on another planet.

How We Detect Informational Macromolecules

(a) Does the sample (A) Compositional analysis. contain macromolecules? (b) What is their composition? (c) Any evidence of informational ordering?

Esobiology is firmly founded on the isolation of macro-molecular species and their purification before attempts at analysis. Some of the most successful methods are empirical recipes of extraction and precipitation.

More rational techniques include diffusional properties of large molecules, free diffusion, sedimentation, dialysis, molecular sieves, and electropheresis, in principle also vapour phase diffusion (to remove monomers)-molecular distillation, gas chromatography and mass spectrometry. Solution chromatographic methods may also rely on the coincidence of functional groups on one molecule, for

example, a polyelectrolyte.

Similar principles underlie non-separative methods of detection which have not been extensively developed to date. Rotational relaxation times can be measured by flow or electric birefringence, or the analogous polarization of fluorescence. Polyfunctionality is tested by intermolecular interactions of adsorbed dyes (for example, optical shifts in acridine orange on DNA) or the monomeric units with one another in special cases (hypochromicity of DNA, diagnoschle on heat-denaturation). More direct chemical tests for polyfunctionality also suggest themselves.

The previous methods, in so far as they lack perfect generality, may give only a clue as to the composition of the macromolecule, as well as its molecular size. At the other extreme, we would seek the complete primary structure to emulate the recent tours de force of chemical technique. Reasonable inferences might be drawn from less complete evidence of structural individuality, hard to evaluate in advance: homogeneity in molecular weight or end-group analysis, crystallinity, or sharp fractionation by any other procedure. A sharp X-ray diagram of a heteropolymer sample could imply its individuality long before it had yielded to full analysis.

Other partial measures of great utility include the seission of the polymer by specific reagents, especially enzymes, to give a pattern of characteristic fragments (the polypeptide 'fingerprint').

The underlying generalization is 'molecular speciation'. Chemogenic synthesis of macromolecules should generate a continuum of nearly equiprobable forms. Biogeny chooses a few of these and generates a sharply discontinuous polydiscrete spectrum, that is, it speciates. Speciation can be discerted by many measures, for example, the distribution of molecular weight. Thus a sample under analysis by a sophisticated instrument might reveal a sac of h. m-polypeptide, containing about

Function Complex with Example Incipient polymer (same species) and polymer-building monomer Incipient polymer (different species) and polymer-building monomer Formed polymer, similar species Auto-replication DNA: DNA + deoxynucleo-side triphosphates Hetero-replication DNA : RNA + nucleoside triphosphates Morphogenesis Collagen: collagen sub-units fibres, mem-branes, vesicles Enzyme Substrate—catalytic effect Cofactors—to form holoenzyme Analogues—complexes inactive, qua enzyme Whatever Neutralizing Antibody: antigen, that is, any chemical species foreign to the reacting organisms crum albumin; hormones Transport Hormones, toxins, nutrients toxins Permeases: nutrients and metabolites for transport in and out of ceffs

a billion (a thousand million) atoms of iron. Virtually all the iron-polypeptide consists of a single species, that is, almost all the molecules have 2,936 carbon atoms, no more, no less. After removal of iron and porphyrin, equal numbers of sub-units containing just C₄₄ and C₇₁₅ are assayed. It would be difficult to escape an allusion to life after a single encounter with the red blood cell that has just been described.

(B) Functional analysis. The adaptive values, the uses that biogeny has discovered for some species of macromolecules, reveal short cuts to their singularity. These functions are all reducible to a structural specification: the stereospecificity of the polymer in reacting with other molecules.

In this list, the enzymatic functions are particularly promising in the light of their specificity and amplifying capability. Many enzymes have turnover numbers of 10⁴ substrate molecules/sec enzyme molecule. If suitable precursors (nutrients) can be defined, integrated enzyme-sequences or metabolic systems, like respiration or photosynthesis, extend the versatility of this approach.

The simpler the level, the more likely are we to find a metabolic analogue on Mars, for example, for the assimilation of elementary nutrients, C. N. O. S or P. into organic molecules. The next more complex molecules, H₂O, CO₂, and O₂ and NII₃, are the most pervasive metabolites of terrestrial life, and the choice among them for searching for evidence of their conversion into other compounds will depend mainly on instrumental considerations. In general, the more complex the metabolite being tested, the less our prior expectation that it was part of an extraterrestrial biogenic system. However, the complete system offers the largest amplification—a single bacterium could grow and multiply into tonnage masses in a few days, but might make the most exacting demands of the environment.

OF TOOR OUALIZE

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Morphology

Biogeny rapidly elaborates higher forms of organization: cells, tissues, organisms, populations, which might be recognizable according to their own forms and to their rectifications of the environment. However, what systematic rules distinguish biological forms in general? Some forms are recognizable, for example, a friend's face, and recognition then contains many bits of useful information. Compound vesicles, apparent cells, are most inescap the in morphogenesis; their absence would at least set an upper limit to the stage of biogeny. Their presence would be extremely provocative, but properly would raise many scepticisms of chemogenic artefact. Nevertheless, esobiology has so many roots in morphology that we could scarcely ignore the insights that our historic practice of it would offer. Any recognizable forms would provoke tangible and hence useful working hypotheses of the Martian system.

working hypotheses of the Martian system.

Some aspects of morphology can be systematized. As an example which might illustrate speciation, ultrastructural spacings in the range of 20–500 Å could be detected by powerful optical (electron microscope, X-ray diffraction) as well as separative techniques. Approaches so cogent to esobial ultrastructure must play an important part in exobiology. Unfortunately, we have little empirical basis to prejudge the morphological detail that might be exhibited by an infra-biogenic planet, since so much of the chemical diversity of Earth has been pre-empted

by life.

As is well known, five-fold symmetries are anathematic in crystallography. Hence, regular pentagonal and lodecal-edral forms might occur as elementary units, for example, perhaps a ferrocene, but no simple law of crystal growth could account for their occurrence in diverse sizes. A glen of periwinkles has a deductively simple signature of life.

Signals

So far I have tacitly assumed that whether or not Mars has achieved biogeny, it has not passed to compageny. Reaction to the once notorious Schiaparellian canali may account for a position which has no rigorous basis. True, we have had no scientifically admissible sign of intelligent activity on or communication from that planet. However, we can only fancy whether an exotic culture would have either the means or the motive to effect recognizable communication. We can generalize that the works of cognogeny would constitute the most startling unlikelihoods, exceptions to biogeny and chemogeny alike.

compactly summarize our scientific culture. For example, a description of DNA and our amino-acids could portray the convergence of physical and chemical ideas in biology, and some of the least predictable aspects of esobiogeny. If we could but do it, a detail of the inter-neuronal synapse and the cytoarchitecture of the cerebral cortex would go even farther. How much of our cognogeny would then be deducible from these facts and our awareness of them?

Purposeful emissions cost enough more than mere listening that we do not undertake them ourselves, but we have made casual efforts to hear them. Further, we might hope to eavesdrop on the internal communications of another planet, perhaps more likely far beyond the solar system. Among other difficulties, efficient information is, by definition, indistinguishable from noise to the unbriefed eavesdropper.

While a rigorous answer to any notions of Martian intelligence is difficult, a realistic policy is not. Cognogeny would reveal itself in divers ways, and, at least for Mars, we have no better recourse than to keep eyes, ears and noses alert for any signs of it as we mak, progressively closer approaches to the planet.

Instrumentation

The rational classification of existing instruments, or those proposed for analytical purposes, is a task as d flicult as it is urgent. The real aim, a classification of possible instruments, requires a total knowledge of physics, and some system for classifying this information that will help us to understand the relationships among existing instruments and suggest new ones. A proposed scan parameter is the energy-level of the transition by which the molecule is recognized. Further parameters include whether photons are introduced or emitted, whether chemical reagents are employed, including auto-reactions, whether the displacement or state of the analysand or of the probe is diagnostic, and, for radiation probes, the role of power, polarization, phase, wave-length, or flux vector of the probe. The first step in a detailed rationalization is to determine whether any more dimensions are needed for our matrix of possible configurations.

Radiation probes are usually limited, either in selectivity—say, absorptiometry, or sensitivity—say, nuclear magnetic resonance—but they have special value in conjunction with chemical reagents. Absorption (power loss) measurements have dominated instrumcatal analysis, Conventional optical methods rarely stabilize or measure input power better than 1:1,000, with corresponding limitations to detectivity. For example, optical molar absorptivity rarely exceeds 10° so that 10-2 molar solutions (6 × 10°2 molecules in a 1-cm-2 cell) would give the lowest useful signal under the most favourable conditions.

By contrast, fluorometric measurement (which can exploit shifts in wave-length, flux vector, polarization and phase) can easily measure 10s molecules and can be extended at least to 10s. The delicacy of excitation methods (which could also include chemical, nucleonic and thermal excitation) stems from the measurement of the data signal merely against a detector noise background as compared with the much larger power fluctuations of practical probes.

Optical activity is also usually measured via loss of power (attenuation of polarized light by a crossed analyser): the molar rotations are relatively small, present detectivity being about 10¹³ molecules. If some method of transforming optical rotation to an excited signal were developed, it would enormously enhance the

power of this technique.

The most sensitive approaches to analysis are two stage mechanisms: the selective displacement of the analysand, then a sensitive detection. In principle, such methods might detect a single molecule, as in mass spectrometry: selective m/e displacement followed by the sensitive detection of an ion that can be accelerated to arbitrary energy. The potential information content of a mass spectrum is especially high since the theoretically measurable mass of a single molecule is defined to a resolution far better than 10-6, independent of the variety of energetic states, which broaden other physical features. Existing instruments still lag behind theoretical limits of mass resolution, yet have already demonstrated their power in organic analysis. Further, the mass datum at high resolution for an intact molecular ion is deductively reducible to a molecular composition, unlike the inferential data given by most other spectroscopic techniques, and the statistics of the fragments also give detailed insight into the complete structure of the molecule. From these considerations the combination of a mass spectrometer with a simple, rugged, separative device, like the gas chromatograph, promises to be the most powerful compenent of analytical systems for biochemistry. However, a science of metrology, the orderly study of methods of measurement, remains to be developed. I can have little confidence that the last word has been said on this issue.

Some Private Thoughts on Exobiological Strategy

The multitude of possible means and detailed ends in exobiology leaves fittle hope that a brilliant flash will illuminate the whole picture as a happy substitute for the diverse paths of esobiology. Nor should there be any discouragement of the variety of talents and insights that would be needed in any event for the full development of the subject. The overriding problem in planning is, of course, how little we actually know about surface detail

and atmospheric composition of Mars. We are also bedevilled by the uncertain hazards, but immense stakes, to either planet of an intemperate rupture of the interplanetary barrier. Earth-based telescopes can, to be sure, add significantly to our present appreciation of Mars, and hence of the hazards of landing. But the next significant step would be a Mars-orbiting observatory, keeping the planet under a constant synoptic scrutiny from a safe distance, close enough to measure significant surface detail, and large enough to maintain the most sophisticated instrumentation, telemetry to Earth, and perhaps even some Earth-based regulation of its surveillance schedule and precautions against accidental intact landing.

Such an approach to Mars would also open the way to political agreements to unify terrestrial strategy and canallow constructive co-operations like the International Geophysical Year of recent history, for example, to facilitate the relaying of synoptic data. While it is essential to mount vigorous instrument development efforts to assure that a landing can ever be implemented. the detailed specifications of experiments should take full advantage of the most up-to-date planetological information. That is to say, the final decision to implement a landing on Mars should be suspended until we can have digested the data from a Mars orbiter. This criterion lends further weight to the strategy of designing a jeneralpurpose laboratory for planetary investigatio... in which many investigators can participate, and which has the flexibility to be readily reprogrammed in the light of new data. At present, for a biologist to participate actively in space research requires a commitment to engineering efforts which few are willing to undertake.

The deliberate staging of the exploration of Mars, perhaps with international agreement to proceed first with reconnaissance while preparations are made for comprehensive landed missions, would allow for the widest participation of interested scientists, both in the design of experiments in exobiology and in the prudent determination of global policy for the solar system.

Some Comments on Instrumentation Design Strategy

for Exobiological Explorations

by

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1. Introduction

The following notes are a first approach towards formalizing a strategy on exobiological instrumentation. These have evolved from many discussions with J. Lederberg and, this last summer, with J. Platt of the University of Chicago, and, in part have appeared in various reports and papers. It is hoped that they will prove useful in making the best use of the very exciting and very expensive opportunities to do exobiological experiments.

11. Search and Analysis

The concerns of exobiology range from simple search for familiar patterns to detailed analysis of the evolutionary history of the planet. The quest for signs of life precedes the effort to understand the nature of the life where signs have been discovered. Different instruments may be most appropriate for each stage.

The difference between criteria for search and analysis is illustrated particularly clearly in connection with the question of intelligent life in other solar systems. At first one has to explore the complete domain of space and possible communications links, asking only for evidence of non-random phenomena unexplainable by known physical laws. A positive response to such a query is not at all convincing as to the question of the existence of intelligent life elsewhere and says nothing of its nature; however, it rationalizes an enormous narrowing of the region of search and thereby allows a corresponding increase in the analytical possibilities. The distinction is also illustrated as follows: If we noticed something moving in a direction different from the wind or, under a microscope, moving in a non-Brownian fashion in a direction other than the fluid flow, we would expect that the "something" was "alive" or propelled as the result of some activity of a living system. We would call that a "sign of life" and could design instruments to use that criteria on a macro or micro scale. Motion would not give much blochemical information, however, and hence such instruments would not be especially useful for analytical purposes. It is clear that a "sign" of life by itself is not sufficient to answer the question of the existence of life, let alone all the questions of exoblology. It must be accompanied by considerable analytical details to give an unambiguously interpretable answer.

III. Evolutionary Epoch

An experiment cannot, by definition, properly be termed exobiological unless it investigates an evolutionary period subsequent to the initiation of the

biogenic epoch (see terminology in Lederberg's "Signs of Life".) Initially, experiments should be directed to the simplest extant organisms. The first reason is rather obvious: evolution must pass through this stage but may have gone no further. A second reason is connected with our ability or inability to design an experiment which tests a general attribute of an evolutionary development. We wish to take advantage of the fact that the simplest species will deviate least from our terrestrial experiences and will include more possible evolutionary pathways than the observation of a specific characteristic of a more complex development. Thus, phosphate-ester metabolism as a measure of the metabolic activity of single-celled organisms includes more evolutionary possibilities than hair as a characteristic of more advanced animal development.

As the complexity of evolutionary development is increased, two additional difficulties arise. At any given time the extant representation of different levels of development are characterized, going from simple to the complex, by increasing molecular weight. The logarithm of the molecular weight ranges from 0 to 2 for the inorganic and from 1 to 6 for the organic stages of chemogeny, from about 8 to 12 for phages and enzymes to 32 for whales in the biogenic realm, and begin at 33 for small attributes of cognogeny. On Earth, one finds that the mean volume occupied by particular constituents of different levels of development is proportional to the molecular weight. In addition, the number of bits needed to characterize the attributes of a given level seem to increase with level roughly as the cube root of the molecular weight. For example, an optical instrument landed on the surface of the earth with a limited lifetime would only be able to detect small organisms because of its !imited field of view. At an increased altitude, with corresponding increase in resolution, it could find larger species. Presently contemplated orbiting telescopes have the field of view but nowheres near the resolution to unequivocally define even the largest artifacts of cognogenic development.

Early landed experiments should thus emphasize the search for molecular constituents of biogenic development, as most suggested experiments have. Another point should be made. As soon as a positive statement can be made about the existence of life on a particular planet there will be a divergence of interests: blochemistry versus social intelligence.

IV. Diversity and Flexibility

No single, unique exobiological experiment can reach all these goals. The experimental opportunities for the next 12 years are the six oppositions of Mars from March, 1965 to December, 1975. The 1965 and 1967 opportunities are associated with what are known as the Mariner missions and the last four with what are known as Voyager missions. They encompass instrument weights from pounds to hundreds of pounds and data link capacities varying from bits/sec to thousands of bits per sec. Viewing all six opportunities as one large experiment, what are the optimal instrument developments which take account of the integrated risk? One extreme of the strategic possibilities is to build all the required diversity and flexibility into one large lander. It would imply postponing a lander until the Voyager series, and preceding it by fly-by and orbiter missions which would serve to narrow both the geometrical volume and the range of possible physical and chemical properties throughout which the lander must search.

One can visualize a lander consisting of many modular instrument components, the permutations and combinations of which could perform a large array of

experiments. The addition of a general purpose computer would enhance in situ analysis and logical decisions. Such a computer -- using microelectronic components, approaching the capacity of an 13M 7090, weighing 32 pounds and with a 900 cubic inch volume -- is already under development. A two way data link, using the transmission capacity contemplated, would permit terrestrial computors and human intelligence to intervene in the lander experiments with policy decisions concerning procedure. Such a concept is not only possible, it should be planned for (see Long Range Program Recommendations). But to believe that one such grand experiment will answer all questions in exobiology is absurd. It assumes a logical completeness of our knowledge of physics and biology far beyond our present understandings. The real question raised by this experimental possibility is where do you start? At what level of risk, at what level of experimental diversity and flexibility is it worthwhile trying the first of a series of lander experiments? This is an extremely difficult strategic question to answer rationally. If, in fact, the choice was to be made to postpone the first lander to the 1971 or 1973 opposition, it would be desirable to plan now for this and reorient the NASA program accordingly.

This choice seems unjustified. It represents an extreme over-evaluation of some of the factors in the various tradeoffs involved. While the philosophy of planning now for such complex experiments in the 1970's is a good one, to start with such complexity overestimates both our theoretical ability and the success of orbiters in narrowing the range of possibilities to be investigated. With too limited a knowledge of the parameters involved, a complex instrument with the total program as well as a greater expenditure of effort for results achieved.

The converse strategy has its proponents: "Take the first opportunity to land an instrument even if it tests a very narrow range of possibilities."

These extremes do not exhaust the reasonable possibilities. We might plan to land an instrument when:

- A. Sterilization criteria can be met.
- B. 10¹ to 10⁵ bits of data can be transmitted to earth accumulated over a 24 hour period.
- C. Five to 10 lbs. of instruments can be landed (not including power supplies, data processing, and telemetry).
- D. Some diversity can be achieved. Certainly more than one test should be included for the presence of microorganisms with enough controls so that a negative answer excludes important possibilities for future experimentation.
- E. It is also reasonable to demand that the experiments would give meaningful results if carried out on Earth under conditions as close as possible to the environment of Mars.

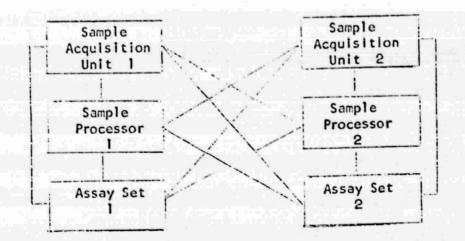
The importance of sterilization in this enumeration cannot be overstated. Uncertainty with regard to meeting any criteria set leads one to add the constraints imposed by b, c, d, and e. Thus, uncertainty with regard to sterilization makes one reluctant to consider the use of probes to gather data about a limited number of parameters.

It is unlikely that one could get experimental verification that sterilization criteria are met for a complete procedure from construction to mission completion, although this might be established for one element of the procedure such as heat sterilization. To generate confidence in a sterilization procedure, it is essential that a sterilization facility be built and actually used in connection with a mission which allows its performance to be tested but in which its successful performance is not critical. An earth orbiter can be designed or an existing orbiter modified to simulate the constraints of a Mars mission; this would allow one to test the success of many of the sterilization procedures in orbit and, in addition, would permit a test of one's ability to make a sterile recovery on earth of a capsule. The success of such an experiment, and the existence of a sterilization facility, whose procedures and efficiency of operation could be observed, would increase enormously the confidence of biologists that the sterilization criteria could be met.

The other criteria for a small instrument payload containing biological experiments for a survivable landed capsule could be met in time to take advantage of the 1967 opposition. In addition, fly-by and orbiter missions should exploit each opportunity so that a complex instrument could provide a detailed analysis by the completion of the Voyager series.

It would be illusory to hope that everything could proceed in such an orderly manner. On the contrary, the unexpected is to be expected: the most vital planning is for the uncertainties that are very much a part of this endeavor. This raises the issue of flexibility. It relates to Individual missions as well as a set of missions. With regard to the latter consideration, it is desired to use the knowledge acquired in one mission to determine the experiments of the succeeding mission. Since there are about two years between opportunities, and the travel time is six months, there is only an 18 month period in which to change plans between missions. This is a very short time. One must plan for the required flexibility by designing a series of instruments or modular components which can be permuted depending on the latest Information acquired. The space craft design must also allow this flexibility particularly since changes in it require the longest lead time. While for any single mission a modular design will add weight and power, when estimated In terms of useful results over a series of missions it might prove most economical. Another alternative would of course be to space the same six missions over twenty years Instead of ten. One should be wary of assuming that this alternative is less expensive. Considering the large fixed expenses in sustaining a program which are independent of the number of planetary shots per unit time, spacing out the program could increase the cost per unit of achievement even though the cost per unit time decreased.

Flexibility is required not only because of new knowledge gained from previous missions, and for a single complex instrument with the logical apparatus to make multiple choices, but also because of unexpected difficulties or even opportunities, due to unplanned variations in payload. Such "emergencies" are an inherent part of today's space science and should be taken account of in the initial engineering concept of a set of instruments. For example, in the arrangement shown in the diagram, two sets of modular units give a large set of payload possibilities ranging from a minimum of two modular units, one sample acquisition unit and one assay unit, to multiple interconnections of all six units.



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A PASTEUR PROBE

A Proposed Experiment

Prepared by Joshua Lederberg

Technical Report No. IRL-1016

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"Cytochemical Studies of Planetary Microorganisms
Explorations in Excbiology"

Principal Investigator: J. Lederberg Program Director: E. Levinthal

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The significance of optical activity for the recognition of life is too well known to require further amplification. As documented in the attached paper by Halpern and Westley, a method is available whereby important metabolites like amino acids can be seemed for optical activity with very high sansitivity, the detection of 100 nanograms being rather easily accomplished (not unreasonably sensitivities down to I managram should be achievable within the general state of the art). This method depends on the coupling of an optically active reagent, such as L-N-trifluoroacetyl-prolyl chloride, to the amino acid ester. If the amino acid is racemic, two diastereoisomers, the L-D and the L-L dipeptides will be formed, and these often prove to be readily resolvable by gas chromatography. The reactions involved are quite straightforward, run smoothly and quantitatively, and can be automated quite readily. The same approach should be easily generalized to other optically active species, organic acids generally, as well as alcohols and amines, and is being explored accordingly, especially for applications to carbohydrates. Besides their abundance and multifarious functions in the cell, carbohydrates have the advantage that methods are available whereby they can be degraded to a unique asymmetric compound. This would make it possible to test the whole genus of carbohydrates for net optical activity without needing to specify exactly which sugar is in question.

The work with amino acids does point to this limitation, namely that if a wide variety of organic molecules are present in the sample, the gas chromatograph would not be easily interpretable, since any two peaks might be related diastereoisomers, or totally unrelated molecules. This difficulty could be circumvented in principle in several ways:

- a. a two-stage separation, the first without the introduction of an optically active probe for example, trifluorescetylation; then each fraction would be tested by resolvability, using <u>D</u> and <u>L</u> respents separately and together prior to the second stage. This is clumsy and may be difficult to implement without recomization.
- b. a single stage reaction run in parallel with <u>D</u>- and <u>L</u>- reagents. This may suffer from calibration problems circumvented by (c).
- c. a single stage reaction with ratio-detection of <u>D</u>- and <u>L</u>-complexes.

 For example, suppose we prepared the enantiomeric reagents with differential labels, for sake of argument say levo-tritium and dextro-C¹⁴. After coupling

to the mixed target material, the product is then chromatographed. For each symmetrical target molecule, the $^3\text{E}/^{14}\text{C}$ ratio will remain uniform in a single peak. However, if an optically active asymmetric molecule is encountered, and gives rise to resolvable diastereoisomers, the tritium will be concentrated in one peak, the $^{C^{14}}$ in another; that is to say there will be a swing in the ratio of the labels to one another. If the target molecule is recemic, two peaks will also be formed (one containing L-D plus D-L; the other L-L and D-D) but the label ratio in each peak will remain constant. Therefore, even when a wide variety of substances may be present in the sample, the ratio recording of the chromatograph output will be influenced only by optically active species. Overlapping peaks will interfere only insofar as they attenuate the shift in ratio by diluting the difference in label.

This approach therefore requires the fewest assumptions about the specific molecules being sought; naturally, there are technical considerations on the choice of a variety of reagents and columns best suited for different classes of substances.

Tritium and C¹⁴ were mentioned as differential labels only for purposes of illustration, though they might well serve for certain purposes. With halogenated reagents, only one radioactive isotope may be needed, electron capture and other methods giving excellent detection of total material. Other ultrasensitive techniques, such as neutron capture methods, alpha-particle backscattering, and so on, also suggest themselves. The greatest utility might be found from mass-spectrometric detection, e.g., with O¹⁸ labelling of the trifluoracetyl group and thermal cracking of the chromatograph effluent; with careful choice of materials, monitoring the m/m+2 ratio would give a very fast, highly sensitive recording for optical activity; the same instrument without cracking could give the full mass spectrum of just the interesting fraction, i.e., data from which to deduce the chemical nature of the optically active species.

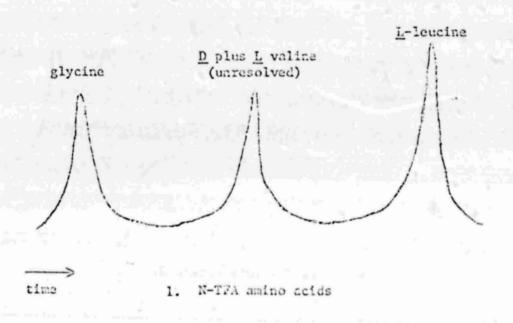
The work already done could be incomporated directly into a useful lifedetecting experiment, namely for the properties of amino acids partly separated by another sub-system. We propose to continue our investigations on refinements along the lines indicated that would yield a system giving a general approach to the detection and identification of minute amounts of optically active materials.

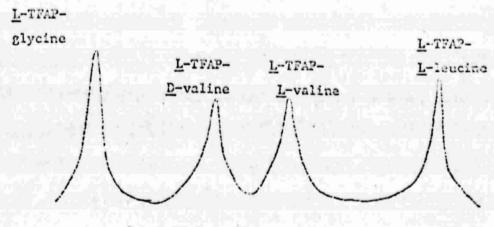
Samples

Our present thinking encompasses the utility of soil samples of the order of 1 to 10 grams, the collection of which would involve a subsystem commensurate with the complexity of the analyzer (i.e., gas chromatograph, mass spectrometer and thereon). The reagents and their handling should be manageable within a kilogram and a power requirement very small compared to the analyzers. These should be no problems of sterilization of these reagents. If means of scaling down these analyzers are found, samples of the order of 1 - 10 mg might plausibly be expected to yield an interesting result.

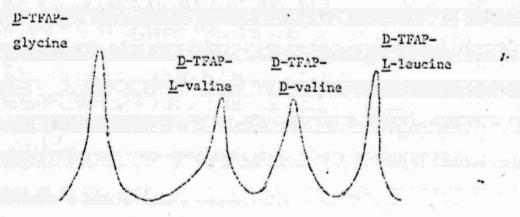
Collection of volatiles from the atmosphere also deserves consideration, perhaps with the help of morning dewfalls. It should be pointed out that on the permafrost model, most of the 'volatile' material of Mars will be distilled or leached out and fossilized at some depth beneath the surface in equatorial regions. Subarctic zones roughly at the times of waves of darkening have the best chance of surface exposure, but digging at the equator should give a similar result - as would shaded cravices. Microorganisms might be expected to be most abundant between the surface and the permafrost and especially in the vicinity of such crevices.

Consider a mixture of glycine (symmetric), <u>DL</u>-valine (racenic) and <u>L</u>-leucine (optically active) in equal amounts of each isomer. The following chromatograms would be realized. (TFA stands for trifluoracetyl; TFAP stands for trifluoracetyl-prolyl.)





2. L-TFAP peptides



3. D-TFAP peptides

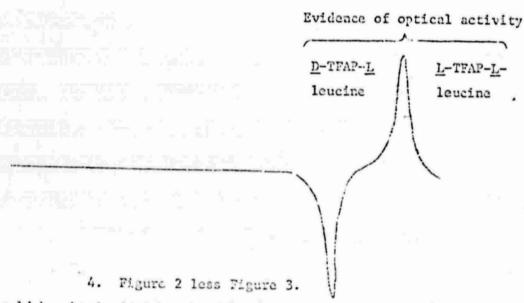
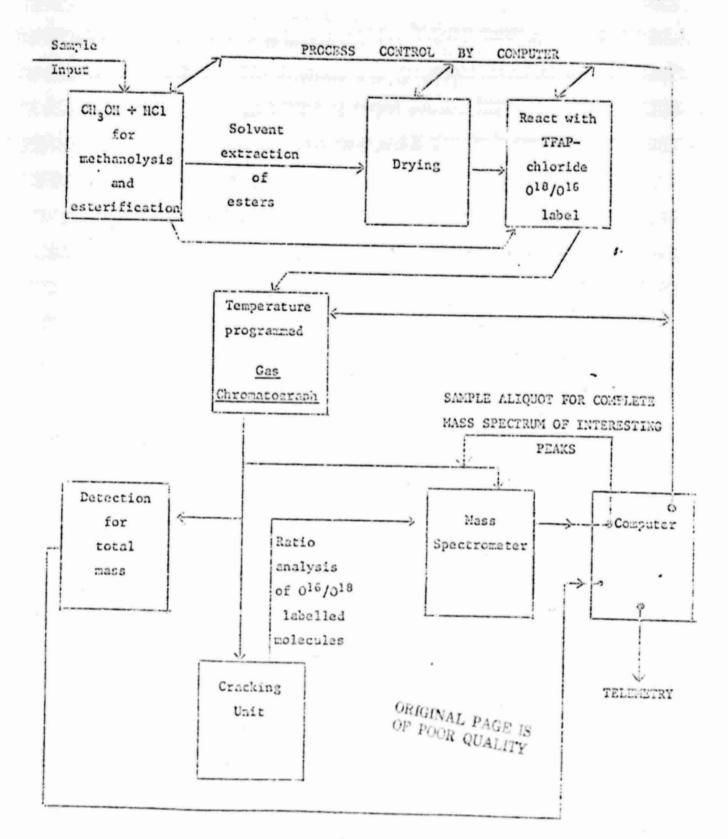


Figure 4 could be obtained either by (b) running parallel columns precisely calibrated and controlled with respect to the reaction with TFAP or (c) differential labelling of the TFAP used in a single reaction.

FLOW DIAGRAM

A Complete Subsystem for the Automated Biological Laboratory.

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OPTICAL RESOLUTION OF D.L AMINO ACIDS BY GAS

J. Perc

CHROMATOGRAPHY AND MASS SPECTROMETRY

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The significance of optical activity for the recognition of life and hence its utility for biochemical exploration needs no elaboration (Lederberg 1965), (Ulbricht 1962). Recently, a number of gas liquid chromatographic (G.L.C.) procedures have been developed, whereby important metabolites, like amino acids, can be scanned for optical activity, with very high sensitivity (Gil-Av., Fischer and Charles 1965), (Halpern and Westley 1965 a,b), (Pollock 1965). The same principle can be generalized for complex mixtures by ratio-detection of D- and L- input reagents when these form resolvable diastereo-isomeric complexes with the target material. We now show the use of mass spectrometry for the ratio-detection, as well as to identify the optically active species.

For this purpose we prepared an artificial mixture of D and L enantiomeric resolving agents, in which the L reagent was labelled with 2 deuterium atoms (L*). After coupling with the target material, the product was gas chromatographed and the peaks collected and passed into a mass spectrometer. For each symmetrical molecule (e.g. glycine), the D and L reagents are unresolved and the label ratio will remain uniform through the peak. However, if an asymmetric molecule is encountered, which gives rise to resolvable diaster-eoisomers, the deuterated reagent will be concentrated in one peak, distorting the ratio. If the target molecule is racemic (D L), two peaks will also be

formed (one containing L*D plus D L; the other L*L plus D D); but the label ratio in each peak will remain constant, We chose trifluoracetyl-thiazoli-dine-4-carboxylic acid chloride as the reagent, because both enantiomers are available (Ratner and Clarke 1937), and deuterium can be incorporated into position 2 with deuteroformaldehyde. Also mass spectrometric fragmentation patterns of its condensates with amino acid esters yielded characteristic peaks which could be used to identify both the reagent and the amino acid (Figure 1).

Figure 1

MASS SPECTRAL FRAGMENTATION OF TFA-THIAZOLIDINE-4-CARBOXYLIC ACID CONDENSATION PRODUCTS

Fragment a n/e 184

In a typical assay, the amino acid sample was esterified with thionyl chloridemethanol and the excess reagent and solvent removed. An excess of the resolving agent (L* plus D) in an inert solvent was added to the residue and the suspension neutralized with triethylamine. After washing with water, the solution was injected into the gas chromatograph and the emerging components collected for introduction into the mass spectrometer. By monitoring the ratio for fragment (a) [184:186] as well as the ratio (b:b+1) for the base peak (M-156] a fast sensitive recording for optical activity was obtained. In addition, the position of the base peak was also used to confirm the identity of the optically active amino acids present (Table), (Figure 2).

TABLE: MASS SPECTRAL MONITORING OF G.L.C. FRACTIONS

CORRECTED FOR ISGTOPIC ABUNDANCE. (*)

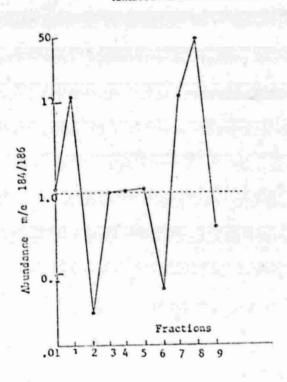
G.L.C. Fraction	Ratio(a:a+2) m/e 184:186	Fragment(b)	Ratio (b:b+1)	Molecular Weight (b+156) and Iden- tity of Amino Acid	Optical Identity of Fraction	
1	28:2.5	158	100:8.5	314-slanina	L	
2	1:24	158	4.5:100	314-alanine	5 _L	
3	38:41	144	97:100	300-glycine	DL	
4	55:55	172	100:98	323-aminobutyric	acid DL	
5	33:32	172	100:98	328-aminobutyric	acid DL	
6	2.5:33	200	8.5:100	355-leucine	D	
7	23:2	200	100:8	356-laucine	D	
8	100:2	184	100:5	340-proline	L	
9	12:31	184	12:100	340-prolina	L	

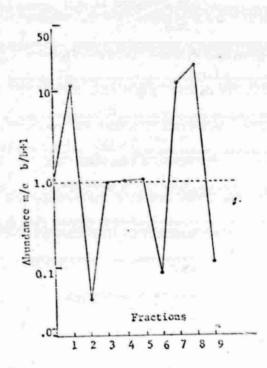
*G.L.C. analysis were carried out on a Wilkens 600C Aerograph, fitted with a micro collector and using a 5' X 1/8" S.S. column containing 5% SE 30 on chromosorb W. The separation temperature was $180\,^{\circ}$ C and the N₂ flow was $28\,$ ml/min.

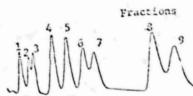
Mass Spectra were determined on a Bendix-Time-of-Flight Spectrometer and the collected sample fractions introduced directly into the ion source.

The utility of mass spectrometric detection, thus substantiated, points to a general method for the speedy, facile detection and identification of minute amounts of optically active materials. Hardware for direct coupling of the gas chromatograph to the mass spectrometer (Gohlke 1959, 1962), (Ebert 1961) was not yet available to us for this study. However, the results of other

Figure 2 ANALYTICAL RESOLUTION OF LABELLED INPUT REAGENT







Gas Chromatograph*

*G.L.C. Fraction:

- D reagent-L-alanine
 L* reagent-L-alanine
- 3. D reagent-glycine and L* reagent-glycine
 4. D reagent-L aminobutyricacid and L* reagent-D aminobutyricacid
 5. D reagent-D aminobutyricacid and L* reagent-L-aminobutyricacid
 6. L* reagent-D leucine

- 7. D reagent-D leucine
- 8. D reagent-L proline
 9. L* reagent-L proline

workers suggests that the technique should thus have a sensitivity in the submicrogram or nanogram range, rendering it useful for the monitoring of metabolic reactions as well as the identification of accumulated asymmetric metabolites.

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Indeed we began our work on the latter tack, and have made substantial progress in the discovery of new means by which to study the DNA of a prototypical organism, Bacillus subtilis, under conditions of adaptation. For example, it is possible to cut the total DNA into about 300 fragments of molecular weights from about 1 to 10 million each, by the use of a specific enzyme called R-1. (this enzyme sees only special codewords in the DNA sequence). In favorable cases, the DNA can be separated by size with agarose-gel-electrophoresis, and some of the DNA fragments can be obtained virtually pure. We have been studying adaptations that involve the DNA sequences themselves, which alter the recognition-words for the enzyme, and thereby change the sizes of the DNA pieces. These seem to occur with remarakable frquuency, and the new DNA pieces thus obtained result in puzzling, but dramatic, changes in the genetic constitution of the bacteria. In pursuing these studies, we must make routine use of electron microscopy to visualize the DNA pieces. We are also attempting to introduce synthetic, monotonous sequences of DNA into the bacteria, but so far without clearcut success-the ways in which the bacteria resist this intrusion are still obscure but of the deepest interest.

More recently we have also been addressing the question of the fundamental limits to the reproduction rate of bacteria--presumably related to the limiting rate of DNA syn= thesis. Claims in the literature of doubling times of 10 mins. (at 37°) cannot be substantiated. We are looking for the "fastest bugs in the world" by selection from natural sources, and so far have found many that approach 21 minutes in synthetic media, but none faster. We will continue to look for "fast bugs" from appropriate natural sources with efficient selective methods, and also for "faster" mutants that may arise in the laboratory. The strains we have isolated are already very interesting as convenient objects for laboratory microbiology -- the conventional strains grow 2 or 3x more slowly, with corresponding impediment to the rate of research; and it is inevitable that such organisms will also have other practical and industrial applications. The examination of these growth-capable strains will be coupled with the study of the molecular mechanisms that make their rapid growth possible -- considerations of some theoretical importance for predicting behavior of exobiological subject material, as well as for the potential engineering of organisms for useful tasks in various habitats.

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This is a non-technical and brief description of the work we are doing in the Genetics Department of Stanford Medical School which is supported by NASA,s Office of Planetary Science under grant NGR 05-020-004.

The search for life on Mars, the next step of which will be carried out by the Viking Mission, shares common goals with laboratory explorations and meteorite analyses which attempt to elucidate the origin of life on earth. Theories of the origin of the planets, astronomical observations, and laboratory tests all support the idea that the primitive atmospheres of many planets would lead to the formation of molecules on the surface of those planets which would resemble those molecules which are the fundemental building blocks of all life here on earth.

There is subtle difference, Because these molecules include the atom carbon, which can atttach to itself four other atoms or groups of atoms, there are often two ways of making these molecules. These two ways lead to two kinds of molecules which are mirror images of each other, much like the resemblance of the left and right hand. When such molecules are formed by primitive planetary atmospheres, or in the laboratory, they are generally racemic mixtures of these molecules, namely equal mixtures of both left and right handed versions. However in living systems, at least here on earth, because of the way they reproduce and make copies of molecules, they most often are found with largely either only the left or right hand versions of these molecules.

Much of the work of our laboratory involves research and development work on chemical, instrumentation, and computer methods of searching for and understanding these subtle differences. We have applied these techniques to the study of meteorites and return samples from the Applio lunar missions, We are finding exciting applications to the study of human body fluids, such as urine and blood, searching for signs of genetic diseases. The instruments we use most often are gas chromatographs and mass spectrometers. Gas chromatographs are instruments which seperate complex mixtures of compounds into their component parts so that they can be analyzed more easily. The mass spectrometer breaks the large molecules we wish to study into pieces and produces signals showing which pieces are present. From this puzzle the chemist can discover the nature of the large molecule from which these pieces came. Because on Mars we will want to do these studies remotely, we work on ways to make a computer manage these kinds of instruments and interpet the results. Interpeting the results requires that the computer carry out the reasoning processes of a very good chemist when it tries to unravel the complex puzzle generated by the mass spectrometer. In trying to solve this very difficult problem of computer science our laboratory benefits by a very intimate collaboration

with the Stanford Departments of Computer Science and Chemistry.

These computer techniques also have their medical applications, Human body fluids are complex mixtures of many large molecules. To screen many samples looking for disease or the affects of possible dietary or atmospheric pollutants on our body metabloism, would be too formidible and expensive a task to carry out without the assistance of a computer. The NASA supported space-related work on these applications of computers has led to a very large project we are now engaged in called SUMEX-AIM. This project has as one of its major goals, the applications of computerized reasoning in medicine. This use of computers to solve problems is known as Artificial Intelligence.

Attached to this brief summary of our work is an article to be published shortly in the magazine Stanford M,D. which outlines the history of our laboratory and explains its research work and other efforts of Professors Lederberg and Levinthal related to the exploration of Mars, Also attached is a brochure describing the SUMEX-AIM project.

ATTACHMENT 36

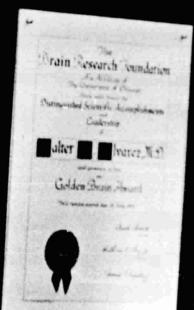
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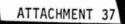
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Report from the President

SHORTLY AFTER BEGINNING MEDICAL SCHOOL at Stanford, our first-year class was invited to the home of Dr. Russell V. Lee for an evening of fun and frolic. As freshmen, we were bewildered by many things—the heavy academic load, long study hours, biochemistry—so such an invitation was especially welcome. Dr. Lee fed us well, entertained us with anecdotes, and then spoke seriously. He said, "You will find that one of the greatest privileges in each of your lives is being a physician, especially one trained at Stanford." At that moment the prospects of having enough energy to finish the first year seemed dim, and that of ever becoming a physician too distant to imagine.

Most of us today feel the truth in Dr. Lee's prophecy, and we have an abiding feeling of privilege in being physicians and having been educated at Stanford. This feeling is quietly personal, seldom mentioned, and certainly not to be misused. With such privilege, however, must go a sense of responsibility to one another as physicians and to the Medical School. To have the School prosper and thrive by producing better and better physicians is a fine compliment to each of us. In this age of cynicism, pointed so often to our institutions, it becomes especially important that we muster and sustain a spirit of enthusiasm.

Our school is ideally located, beautifully equipped, and staffed by superb scholars. The hope of virtually every medical student entering Stanford is still to be-



come a fine physician. Today he is more privileged than ever because of the incredible competition simply to gain admission to a School of Medicine, *particularly* Stanford.

If this student is, in fact, to feel this sense of privilege and mutual regard, there must be a substrate atmosphere that engenders such a spirit. We, as alumni, must be at the base of this spirit and must feed back to the School as much enthusiasm as possible.

There are tangible ways to express our feeling of privilege by sustaining a high regard for one another and the School. If there is a diminishing feeling of regard, then the reasons for it should be made known to the Alumni Association and changes should be made. As physicians and alumni, we should keep our lot vigorous, responsive, and constructive to the continued properity of the School. So, let us not be consumed by cynicism, but rather strengthened by enthusiasm.

ROBERT E. BERNER, M.D. '44

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Stanford M.D.

STANFORD MEDICAL ALUMNI ASSOCIATION

Fall 1974

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ON THE COVER: The walls of Dr. Walter Clement Alvarez's office testify to his long service as author, editor, syndicated columnist, historian and philosopher, as well as authority on diseases of the digestive tract. An interview with the last surviving member of the Class of '05 begins on page 2.

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Walter Clement Alvarez: Physician Extraordinary

In this interview, a famous physician-philosopher talks about shrewd diagnosticians, little strokes, mindless medicine, and doctors who use long words.

HE FOUR-STORY BUILDING, its exterior charmingly decorated in black marble, is an anachronism to the huge modern skyscrapers towering over it on Chicago's busy Michigan Avenue. But at the top of some long stairs, closed temporarily for repairs, is an office. You reach it by a small elevator instead and are greeted by two polite secretaries who announce, "The doctor is expecting you." You enter and find a man seated in a leather chair before an old wooden desk, cluttered with manuscripts, journals, and newspaper clippings. You look around and see walls bedecked with impressive diplomas, books, awards, and photographs.

However imposing this image may be, it takes just a moment to discern disorder everywhere—books, journals, and old newspapers stacked on chairs, on tables, and on the floor. You get the feeling you didn't just walk into a doctor's office, but a scene from Ben Hecht's Page One.

The man behind the desk is Walter Clement Alvarez, M.D., an internationally known authority on the physiology and diseases of the digestive tract, former medical school professor, author of many books for the professional and the layman, famous syndicated newpaper columnist, historian and philosopher, editor emeritus of *Modern Medicine*, and last surviving member of Cooper Medical College, Class of 1905.

Alvarez turned 90 last July. On that occasion the Brain Research Foundation of the University of Chicago bestowed upon him the Golden Brain Award for his distinguished scientific accomplishments and leadership. He is a healthy-looking handsome man, over six feet tall, with thinning white hair, thick rimless spectacles, broad shoulders, clean features, and an engaging smile. Although he looks Scandinavian, his ancestry is Spanish and German. Alvarez speaks with candor.

"I damned nearly hurt myself seriously," he said, pointing to a bruised black eye. "A couple of days ago I fell and my head struck the corner of the desk. The left lens was shattered. It's a miracle I didn't get any splinters into my eye."

He smiled and said, "Do you really want to know what happened?

"I think I had a little stroke. Many people over 50 have them. Nine out of ten of these little strokes are so mild, they leave no residuum," Alvarez said, matter-of-factly. He went on to explain that some 40 years ago he began researching the subject because he recognized doctors did a poor job in diagnosing little strokes.

"I found either nothing or very little in the medical literature," said Alvarez. "In one book I found eight lines. In another book of 600 pages on diseases of the brain I found 10 lines. The subject had, for the last century up to about 1950, been rigidly avoided in medical circles, and strictly tabooed."

In 1966 Alvarez published a book, Little Strokes (J. P. Lippincott), to familiarize readers with what he had learned through a half century of study of the disease. "I should explain," he wrote, "that in the past the patients with this common disease rarely had their troubles correctly diagnosed. This is because they almost never told their physicians of the dizzy, vomiting, blackout, or falling spells which suddenly, one day, worked a distressing change in them."

Why should a gastroenterologist be interested in little strokes? "Actually, any gastroenterologist worth his salt must be somewhat of a neurologist and a psychiatrist," he said. "All my life I have never wanted to be so confirmed a gastroenterologist that I could not sometimes get deeply interested in patients who kept coming in with abdominal discomforts or pains for which no cause could be found.

"I began to ask myself, if so often I keep seeing patients with little strokes—because they thought they should consult a stomach specialist—then all of my gastroenterologic friends must also be seeing such patients. But why do they never talk or write about them in the medical journals?

"With a little thinking I got the answer. The patient with a little stroke that has produced a constant misery in his abdomen rarely thinks to tell his doctor about the sudden appearance of trouble, with perhaps a woozy spell or a fall to the floor. And he rarely mentions the great change in his character and ability. Left to himself he will talk only of his stomachache. Unless the



Ready for a drive to the mountains in the 1930's; Dr. Alvarez with his sons, Robert at left and Luis at right.

gastroenterologist gets a hunch from the man's dull face, or asymmetrical mouth, or poor grooming, or slow wit, and unless he turns to the patient's wife to get the essential story, he will not recognize one little-stroke patient a year."

ALVAREZ'S OFFICE testifies to how often in the past 70 years his instincts have been of service to medicine, patients, and himself. The evidence views the visitor from black frames, hung in tiers on the walls. Alvarez with the brothers Charlie and Will Mayo, Alvarez with the staff of the Hooper Foundation, Alvarez being interviewed by newspaper editors, Alvarez receiving the Friedenwald Medal, Alvarez with friends Allan Gregg, Alfred Kinsey, and many more; while in yellowing photographs a young Alvarez, even then portly and paternal, poses with his wife and children, and in another photograph is shown with fellow hikers on a trip into the Sierra.

Whom does he consider as a major influence in his life and career? He turned around and pointed to a photograph of William Osler on the wall behind him. "I never met Osler in the flesh, but I heard him lecture once in 1913, when I was at Harvard," he said. "But he has always been my hero and my great teacher and inspiration."

Soon after his graduation from Cooper Medical Col-

lege in San Francisco he bought Osler's Aequanimitas and Other Addresses, a book Alvarez recommends as must reading for all medical students.

"Osler reinforced in me the idea that medicine is an art, not a trade; a calling, not a business," the doctor said. "In an old diary of mine written in 1906, there is a note showing that I was already wondering how I could so work and grow mentally and spiritually as to be of the most use to humanity and to my profession.

"I learned from Osler that a fine physician should be a well-educated man, with much knowledge, not only of medicine but also of general literature and the psychology of men and women. I have always believed strongly in Osler's injunction that everyone should 'burn his own smoke,' by which he meant that no one should grouse and keep telling people about his discomforts."

In his many years with the Sierra Club, Alvarez proclaimed, he never made a remark to fellow mountain climbers that it was raining, that he was wet and cold and hungry, or that dinner was late.

Alvarez was also influenced by Osler's chapter on the joys of collecting the old medical books that are classics. This idea started him on a hobby he claims is still giving him the greatest pleasure. Over the years his library grew, and eventually it contained one of the



Impressive diplomas, awards, and many shelves of books surround Dr. Alvarez's busy desk in his Chicago office.



STANFORD M.D., Vol. 13, No. 4

finest Darwin collections and most of the books written by the founders of American medicine.

"I'm convinced that any man who hopes to be not only a leader in his specialty but also a good and interesting teacher, lecturer, and perhaps editor should know well the history of his subject's development," he said.

Alvarez said he liked Osler's idea that a physician should so live that, eventually, he will become a philosopher. "In many ways I soon did become a philosopher," he remarked, "each day helping my patients with their life problems."

A second important influence in his career was Dr. Emile Schmoll in San Francisco, his Stanford professor with whom he went into partnership in 1910 and completed his training in internal medicine.

"He was a shrewd and clever diagnostician." Alvarez recalls. "He taught me to use my eyes and ears as he did. He would take me out into the long corridor of the City and County Hospital, and as patients limped by, he would ask me, 'What is that curious waddle due to?' or 'What is that limp due to?' And he kept doing that until eventually I knew many types of diseases at a glance."

Such experiences, said Alvarez, have caused him to beg some deans of medical schools to urge their teachers not just to lecture about diseased people, but to show them to their students as well. He also thinks more effort should be made to show medical students patients who have no organic disease.

"When I was in medical school, no professor ever showed our class a patient with a common nervous disease such as migraine or hysteria," he said. "And yet when I graduated and started to practice, migraine was one of the commonest diseases I had to deal with. No one in college had ever told me about a little stroke, but when I went to the Mayo Clinic I saw patients with little strokes every week. No one had ever told me about nonconvulsive epilepsy, yet when I had learned to recognize the symptoms and signs. I saw these patients by the hundred.

"I feel that if we teach our medical students to recognize such patients, and more often to use their eyes and ears in making a diagnosis, fewer mistakes will be made."

Alvarez fears that many medical schools nowadays are turning out poor diagnosticians. "Today many a resident, like many of us internists, wants his diagnosis made for him by a laboratory girl or an X-ray man," he explained. "Worse yet, he often orders dozens of tests—often aimlessly and with no thought of what they can show or what they will cost the patient who hasn't much money.

"Another trait of many residents which worries me is their great love of what is spectacular in medicine. They want to learn to make liver biopsics, to perform gastroscopies, to catheterize the heart or to perform angiography or culdoscopy.

"It might be preferable to see them learn the art of diagnosing with a good history and a good physical examination."

Alvarez's eyes sparkled with enthusiasm when he described how an assistant many years ago had presented him a patient referred by another doctor for consultation. According to the original diagnosis, the patient had a chronic diarrhea due to amebiasis; but since treatment with amebicides had not quieted his bowel, the resident reasoned that if parasites were really present, they had been too few to cause any symptoms. "What the patient has is a hand so warm that he must have a toxic thyroid gland," the assistant said.

"I could have hugged that resident because he was using his powers of observation and thinking—and he was dead right," said Alvarez. "Two days later, when a surgeon removed a small but highly toxic goiter, the diarrhea quit and the man was well and happy."

Weaving in and out of Alvarez's conversation are ideas and concerns that have been central to him throughout his professional career—the notion, for instance, of purging all patients before operations. When he was still an intern at City and County Hospital of San Francisco, he tried to get surgeons, including his chief, Dr. Emmett Rixford, to stop the ancient practice. "Why do you give everyone 10 grains of jalap, plus 10 grains of calomel, plus an ounce of magnesium sulfate?" he asked, incredulously.

Young Alvarez argued that such a practice resulted in weakening, dehydration, and exhaustion of the patient, and was often the cause of postoperative death, "My surgical teacher, instead of firing me as a nuisance, admitted that he didn't know why the patients was purged," Alvarez said. "Day after day I kept showing him that when some of the older and weaker patients came up to the operating room after a sleepless night on the toilet, they were so worn out that they were easily thrown into shock, and finished off by the operation.

"Then I went to the library, and after weeks of reading, I discovered that the practice of purging patients was a remnant of the idea of many primitive peoples that when a lad is to face an ordeal, such as torture incident to his initiation into the tribe, he must be prepared by purgation, fasting, vigils, and abstention from sex."

In 1918 Alvarez published this evidence in a medical journal, and soon many of the surgeons throughout the world gave in, and either quit preparatory purging of patients, toned it down so as to make it less harmful, or ordered only an enema.

"This was one of the most useful things I did in my life," says Alvarez. He went on to discuss what he feels to be a fundamental need in medical education. "It is difficult to understand why in medical schools they teach students to diagnose diseases and to treat them, but no professor gives a single lecture on how to get along with people," he said. "For instance, take the example of patients who must wait in a doctor's reception room for two to three hours, especially when they have been given an appointment.

"If I were a medical school dean, I would like to retain as wise teachers in the school a few elderly physicians who, in many years of practice, had gained great wisdom. I would like the wise teacher to show the students the many advantages of making friends with his patients and never fighting them. I would like to see the old-timer giving the students a few talks on how to start a practice, how to furnish an office, and how to keep interested in the local medical society.

"As I remember, in all my courses of medicine I never heard a word about how to start my practice, how to fix up my office, and get a secretary, and send out bills. Much of this sort of teaching could be accomplished with videotapes."

For many years Alvarez has campaigned vigorously to point out the medical profession's shortcomings, and to persuade doctors to abandon practices they could do without. His talks and writings on this subject also reveal a unique sense of humor.

"I wonder how many of us physicians," he wrote in Modern Medicine, "realize that every so often, while examining a patient, we scare him half out of his wits by 'pointing' like a hunting dog that has just seen a pheasant. Time and again at consultations I have seen my friend, the doctor, when he found a tumor mass or a large spleen, or a large lymph node, show instantly that he had made a great discovery. This, of course, alarmed the patient, but what made matters worse was that the doctor dismissed the person without a word of explanation.

"Obviously, if we physicians must 'point,' at least let us immediately tell the patient what we found, and if it is of little significance, let us make sure we have convinced and satisfied him of this."

Early in his life Alvarez realized that trying to learn to write well and clearly could be a tremendous asset in medicine. "Unfortunately, quite a few doctors today pride themselves on their ability to use long technical words," he said. "They will always say cerebrovascular accident for a stroke, and urticarial wheals for hives.

"Many of us who write in a ponderous way, using long and obscure words, are fooling ourselves into believing that we know a lot, when really we don't. I've always believed my chief was wrong in thinking that by translating a patient's complaint into Greek, he had thrown a great light on it!"

Alvarez said this is especially true of psychoanalysis

which, "with its sometimes almost unintelligible gibberish, is a manifestation of the present-day worship of the unintelligible." He said he scoffs at the writings of leading analysts because "they write unintelligible gobbledegook."

Freud, according to Alvarez, should be seen for what he actually was—an able former scientist who became a gullible person, a mystic, a dreamer, a maker of fantasics, and a man who found it easy to convince himself that a fantasy that had come into his mind was a "world-shaking" fact.

ALVAREZ WAS BORN IN San Francisco on July 22, 1884. His father, a native of Spain, was at that time attending medical school. He later became a government physician in Hawaii where young Walter lived with his family until 1901, when he embarked on a medical education at what became Stanford University School of Medicine. In 1910 Alvarez went into partnership in medical practice with one of his professors and completed his training in internal medicine.

In 1913 he did research at Harvard University under the great physiologist, Dr. Walter B. Cannon. From 1913 to the end of 1925 Alvarez practiced internal medicine in San Francisco in the afternoons and did research at the University of California. By 1925 he had become an associate professor at the University.

Later that year he accepted an appointment at the Mayo Clinic as a consultant in internal medicine. For the next 25 years he carried on much research in both laboratories and clinics, becoming internationally known as an authority on the physiology and the diseases of the digestive tract. In 1934 he became professor of medicine at the University of Minnesota (Mayo Foundation). He was gradually recognized for his role in emphasizing to physicians the influence of the mind in producing symptoms of disease, the significance of allergy, and the fact that many persons as they grow older suffer small, unrecognized strokes which become cumulatively more important.

Because he was outspoken and criticized many clinic practices, Alvarez said he never felt "a member of the Club" at the Mayo Clinic. "When talking to an audience about some clinic practice, I unconsciously would say, 'they' do so and so, and not 'we,' "he said. "Dr. Will Mayo sensed this, and with his usual homely wisdom and kindness he tried to comfort me. He said, 'Don't worry, Alvarez; a rat has to live in a strange garret for a good many years before he is accepted as one of the gang!"

Alvarez has long been a student of the history of medicine and has been much interested in human genetics, collecting a library of over 800 books by persons who have been insane, crippled, blind, or otherwise handicapped by illness. He recently donated this entire collection to the Mayo Clinic Library.

He has written more than a dozen books, including The Mechanics of the Digestive Tract (Hoeber 1922), Nervous Indigestion (Hoeber 1930), Diseases of the Digestive Tract (Oxford 1933), An Introduction to Gastroenterology (Hoeber 1940), Nervousness, Indigestion, and Pain (Hoeber 1943), The Neuroses (Saunders 1951), Danger Signals (Follet 1953), Live at Peace With Your Nerves (Prentice-Hall 1958), Practical Leads to Puzzling Diagnoses (Lippincott 1958), Minds that Came Back (Lippincott 1961), The Incurable Physician, An Autobiography (Prentice-Hall 1963), Little Strokes (Lippincott 1966), Nerves in Collision (Pyramid 1972), and Homosexuality Vs. Gay Liberation (Pyramid 1974).

His career has included the editorships of the American Journal of Digestive Diseases, Gastroenterology, GP (The Journal of the American Academy of General Practice), and Modern Medicine.

After leaving the Mayo Clinic to retire in Chicago in 1950, Alvarez was persuaded to write some 15 booklets designed to help laymen understand their diseases, and began to syndicate these booklets in many newspapers. The material was so well received that The Register and Tribune Syndicate signed him up to write four columns a week for them. Before long his column was in more than 80 papers in the United States and Canada, and 20 more papers scattered all over the world. Soon he was receiving over 100,000 letters a year from people who were asking him for help and advice.

"I sensed at first that some physicians doubted if my writing for the lay public was quite 'ethical,' " he said. "But soon most of them seemed to understand what I was trying to do. One ophthalmologist in a city of 100,000 told me that a column of mine on eyes had caused over 100 people to come to him to see why their vision was failing, and several had come just in time to be saved from blindness."

Alvarez's career is replete with honors from scientific and professional societies, including the Friedenwald Medal, the highest honor awarded by the American Gastroenterological Society. He belongs to 48 medical societies and has been made an honorary member of 30 professional and scientific organizations, among them the Royal Society of Medicine of England, the Gastroenterological Society of Paris, and the National Academy of Medicine of Spain.

Throughout his life Alvarez has found time, despite the heavy demands of his scientific, medical, and editorial work, to enjoy hobbies and peripheral interests. In his youth he enjoyed mountaineering and photography and ever since he was a boy he has read widely in archeology, biology, general science, travel, and biography. He reads rapidly in five languages and has found time to travel and lecture throughout the world.

Alvarez has been blessed in his heritage and in his own family. In 1907 he married Harriet S. Smyth of Berkeley, who was at his side until her death last year.



Alvarez, his wife Harriet, and their children, circa 1915.

Harriet and Walter had four children, now married and living in California. A son, Dr. Luis W. Alvare.., is a professor of physics at the University of California at Berkeley. He won the Nobel prize in physics in 1968.

At 90 Alvarez is busier than ever in his life. "I still see a few patients—mainly old friends," he said. "And there are still several books, for the making of which I have gathered facts, and now I hope I can live long enough to write them."

He spoke of loneliness, mainly at night. "But I'm happy to say that I'm still full of enthusiasms. I would hate to be without them—they make life so much more worth living.

"I think life has a purpose when a man keeps trying all his days to leave the world a little better than he found it. Also he is happy if he can be doing each day something creative—making something that is of value."

-Spyros Andreopoulos



Nix Olympica, gigantic volcanic mountain on Mars, as photographed by Mariner 9 in 1972. The mountain is 310 miles across at the base, and a main crater at the summit measures 40 miles in diameter. Nix Olympica is more than twice as broad as the most massive volcanic pile on Earth.

Are There Microbes on Mars?

Stanford scientists helped define the life detection tests that will land on Mars, and along the way are adding to our knowledge of life on earth.

T IS A MAJOR ENIGMA that has confronted philosophers for thousands of years: does life exist elsewhere than on earth? The Greek philosopher Metrodoros of Chios in the Fourth Century observed: "To consider the earth as the only populated world in infinite space is as absurd as to assert that on a vast plain only one stalk of grain will grow."

Since Dr. Joshua Lederberg, chairman of the Department of Genetics at Stanford University Medical Center, coined the word "exobiology" more than a decade ago, the Stanford University School of Medicine has taken an active part in the attempt to solve that enigma—and along the way has gained additional knowledge of human life itself.

"Mars: the planet fourth in order from the sun, conspicuous for the redness of its light," says Webster. Although the School of Medicine cannot take credit for the color, it has had an important share in the explorations of that planet. In 1972, for example, Drs. Lederberg and Elliott Levinthal, director of the Genetics Department's Instrumentation Research Laboratory, benefiting from collaboration with Dr. Lynn Quam of Professor John McCarthy's Artificial Intelligence Laboratory, took active parts in the vastly successful Mariner 9. That satellite orbited Mars for several months, mapping more than 85 percent of the surface, and recording for the first time a great variety of geological forms. "Mariner 9 gave us enormous insight," says Lederberg. Wall-size computer-processed pictures obtained by that satellite decorate the hallway outside the Instrumentation Lab on the ground floor of the Medical Center.

The next step, which the National Aeronautics and Space Administration calls the Viking Mission, will attempt to soft-land two automated science payloads on Mars. The plan calls for two spacecraft to be launched from Kennedy Launch Center within a month of each other in mid-1975 and cruise 400 million miles through space for nearly a year before reaching their destination. Then lander instruments will examine the surface for microorganisms, organic compounds, minerals, quakes, magnetic dust, and gases. The general objective, according to NASA, is to "obtain scientific data which will significantly increase our knowledge of Mars, with particular emphasis on providing informa-

tion relevant to life on the planet." Dr. Lederberg is one of seven eminent scientists on the Biology Team which has devised tests for life based on photosynthesis, metabolism, and growth. Dr. Levinthal is one of seven members of the Imagery Team which has devised a stereoscopic pair of cameras to scan the surface and skies.

Stanford's involvement in the Mars missions dates back to December 1959. Then, with the help of a small starter grant from the Rockefeller Foundation, and later with extensive support from NASA, the Department of Genetics entered the field of exobiology—laying the groundwork for the experimental study of the existence of characteristics of life that may have evolved on other planets.

Speaking in 1960 at the first international space science symposium in France, Lederberg noted that of the other bodies of the solar system, Mars presents the highest probability of sustaining forms of life comparable in any way to those we recognize on earth. Particularly, he called attention to the presence of appreciable amounts of carbon-containing compounds such as those from which life on earth is constructed. Lederberg noted that exobiology offers a unique, fresh approach to the problem of the origin of life, and that microbiological analysis affords the most promise for detecting the presence of life. At that time he suggested a microscope-television combination as the most efficient sensory instrument.

The Biomedical Instrumentation Laboratory was founded in 1962, with Dr. Levinthal as principal investigator, in an attempt to design devices to detect life on other planets. NASA helped set up the lab to provide the general experiments for designing missions with the capability for biological exploration, but the instruments began to take on increased purposes in medicine. The early NASA work led to later development of ACME (Advanced Computer for Medical Research) and SUMEX (Stanford University Medical Experimental Computer) and mass spectroscopy—gas chromatography, projects established with National Institutes of Health support. Lederberg emphasized that "we would never have gotten started without NASA help."

How is the exploration for life on Mars an appropriate study in a medical center? Dr. Lederberg cites the



A model of the Viking lander which will be detached by a Viking orbiter to descend to the surface for a variety of scientific experiments.

Dr. Elliott Levinthal (right) and co-worker with a Viking lander test model during trials in Denver this fall. Cylinder in the foreground is one of two cameras; the ten-foot-long arm that reaches out to obtain surface samples is visible in the background.

enormous implications for every science of finding new life forms. "For example, in our basic studies now, DNA is the watchword, but there is a question if that is the only solution for building new organisms. What we would learn if we found life (outside earth) would certainly count in medicine."

There is a further reason: "The know-how of medical science will ultimately determine the success of a life-seeking mission," says Lederberg. "And, on the other hand, the advanced technology supported by NASA is of great importance in medical research. At a more tundamental level, a question such as 'Where do viruses come from?' is as basic to medical science as it is to the exploration of extraterrestrial life."

THE EXTRAORDINARY MOSAICS of Mariner 9 photographs reveal the knowledge scientists have gained so far in their search. Previous "flybys" had suggested a relatively simple surface dominated by cratered plains. Mariner 9 revealed huge rifts that suggest the planet's interior may be geologically alive. There are broad volcanic zones of extensive lava flows, violent winds, giant basins, and, most startling, long channels for hundreds of miles, with small tributaries and high "walls" suggesting water crosion.

Mariner 9 put to rest some of the old visions, such as the famous canals of Schiaparelli and Lowell. They are not there, just as the technological civilization envisioned by Edgar Rice Burroughs is not apparent.

The cameras did see craters—some more than 300 miles across—a rift 2,300 miles long and plunging 20,000 feet, volcanoes larger than any on earth, and Mars' highest point, Nix Olympica, three times as high as Mount Everest. Photos reveal polar layers of dust, volcanic ash, dry ice, and water ice, and shifting sand dunes on crater floors.

Stanford had particular interest in Mars' changing features. For example, a dark area resembling a spear-head appeared 13½ days after an earlier picture showed no such feature. To determine the extent of the darkening, scientists fed the images of the two scenes into a computer at the Artificial Intelligence Laboratory at Stanford. The differences between the two came out in a third picture dramatizing the change. The photos from Mariner reveal as many questions as they answer, however. Are the changing patterns of light and dark the result of dust shifted by winds, or of seasonal vegetation? There are wandering channels. Are they beds of ancient rivers? How much free water is available to possible living systems? How thick are the polar caps?

Mariner 9 recorded winds exceeding 120 miles per hour, with planet-wide and local dust storms. Instruments recorded the effect of atmospheric dust on decreasing surface temperatures. Such data could be valuable for understanding earth's circulation of atmosphere, such as the potential effects of pollution, as scientists try to predict the level of smoke and smog that could trigger another ice age on earth. Mars' atmosphere was shown to be composed primarily of carbon dioxide with small amounts of carbon monoxide and oxygen that result from the photochemistry of a carbon dioxide atmosphere with a small amount of water. And photos revealed that the two satellites or moons of Mars are what they appear to be: old, dark, battered, entirely natural objects.

Experiments relating to life processes have had to await the first soft landing, which Viking hopes to accomplish. The biology experiments for Viking have had a long development. "Our hypothesis is that biological processes led to the origin of life on earth," ex-

plains Dr. Levinthal. "The transmission of DNA, the formation of mutants, the ability to reproduce are properties of molecules that are the same for all life as we know it—biochemical processes that evolved over a long period of time... We are working with a very narrow definition of 'living': just those processes fundamental to a carbon-based life that is like ours: possessing carbon, hydrogen, oxygen, nitrogen, and water." The experiments are based on the assumption that life on Mars would depend upon the same basic carbon chemistry that supports life on earth.

An early experiment devised at Stanford for the lander mission was to find phosphatase using flourescent techniques. All life (as we know it) uses the phosphorus group as an energy storage bond and has some form of the enzyme phosphatase involved with its metabolism. So scientists invented a process to cleave the bond, leaving a fluorescent residue to show that phosphatase was present. In the early 1960s Lederberg, Levinthal and their co-workers became involved in developing the instrument which could best do this. They came up with a device the size of a milk bottle, and called it a multivator (for multiple evaluator). It was designed to suck dust into an opening in its base, and as the dust filtered through tiny chambers it would stick to the walls. Then the chambers would fill with water and test chemicals, like fluorescein phosphate.

Following development of the multivator, the Stanford scientists moved away from the engineering problems in devising life detection systems. At first, teams of scientists were selected by NASA to assist in planning experiments, particularly to define the critical measurements which should be made and to evaluate candidate instruments. Another team became responsible for the construction of instruments. In the case of the Viking camera, for example, the Viking Project Office directs the prime mission contractor, the Martin Marietta Corporation, which in turn directs the subcontractor for camera construction, the ITFK Corporation. Scientists like Lederberg and Levinthal have become more involved during the last ten years in showing concepts of procedures, which another team could reduce to flyable hardware. "Mostly," says Lederberg, "we hope to innovate and test design concepts-an intellectual exercise, so we can make realistic proposals and criticisms."

Probe. an assay by optical activity for the presence of living organisms on a planet. One feature of many biological organic molecules present in all living systems is their optical activity: they votate the plane of a beam of plane-polarized light. Molecules which have this behavior are made of atoms that can be put together in complementary ways, like left- and right-handed gloves. This optical activity cannot be mea-

sured directly because of the small rotation of most biologically interesting compounds such as amino acids and sugars. So the basic criterion is not optical rotation, but asymmetry based on molecular statistics: whether there is an equal number of left- and right-handed versions of these molecules.

A sensitive technique was developed to demonstrate the asymmetry of amino acids, applicable to soil samples or condensates from the atmosphere. The assay could be extended to indicate growth by inoculating Martian soil with chemical compounds, then analyzing the soil for relative abundance of the left- and righthanded versions of the species.

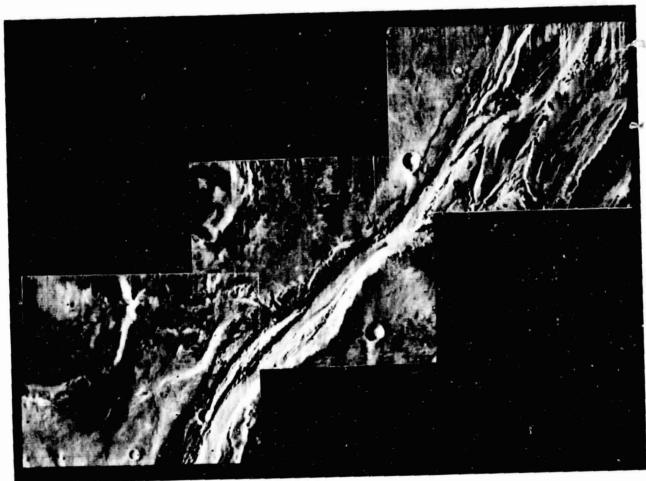
In their paper describing the Pasteur Probe, the Stanford group also noted that "the most powerful instrumentation would combine a gas chromatograph with a mass spectrometer." A soil sample, for example, would be combined with chemical reagents. Then a gas chromatograph is first used to separate the resulting mixture into its constituents by passing a gas through it. The separated portions are then fed into a mass spectrometer, which identifies the individual chemical components. At this point the scientists were thinking along the lines of a "planet-lab," says Levinthal. "This was when we thought the Saturn 5 rocket would be the

launch vehicle, and we could have 5,000 pounds of science. With a lab instead of fixed experiments we could make appropriate adjustments, like changing a temperature."

Such a lab called for computer-controlled instruments like the mass spectrometer-gas chromatograph. Writing in 1967, the Stanford researchers noted, "If characterization of life on Mars is an additional scientific goal, the concept of a computer-managed, reprogrammable, automated biological laboratory is the logical next step.

"Participating scientists would be competing not for payload weight or engineering support, but for prorated time on the computer system. . . . Such a concept allows for the broadest possible national and international scientific participation. The scientist in his own laboratory could direct and evaluate the results of an experiment carried out on Mars. He could initiate

A channel thought to have been formed by running water in Mars' geologic past is seen in this mosaic of three pictures of the planet taken by Mariner 9. This small segment of the channel is about 46 miles long, and the "flow" is northward, from lower left to upper right.



experiments anytime without need of having had to design space-qualified hardware years earlier."

So Stanford became involved in how to design the computer which could act like the scientist. Such a computer could be an important research tool in analyzing medical data or unknown organic compounds on earth, or data obtained from instruments such as the mass spectrometer—gas chromatograph in order to determine molecular structure, or to infer protein structure, with application for treating infectious diseases, for example.

The importance of computers in medicine was increasingly recognized, and early this year Stanford School of Medicine received a five-year federal grant of \$2.75 million to establish the first shared national computer facility for medical research, known as SUMEX. Its primary focus is on applications of artificial intelligence in medicine, employing the computer to reach decisions and solve problems through symbolic analysis and reasoning rather than as a number calculator. Significantly, the support is from the Division of Research Resources of the National Institutes of Health, rather than NASA.

Another spin-off from the NASA research has been the well-known cell separator that Lederberg calls a "resounding success." Developed with grant support from the National Institute of General Medical Sciences, it is the first device which enables researchers to take rare cells from the general cell population for individual observation and separation on the basis of their fluorescence. The device was developed by Dr. Leonard A. Herzenberg, professor of genetics, and his co-workers at Stanford. One of the most important applications is in the study of the body's immune system. helping to determine the chemical and biological characteristics of the different cells, and the specific functions they have. This could lead to possible prevention of organ transplant rejection, and more effective treatment of diseases such as leukemia and hemophilia. Commercial versions of the separator are available now to research teams in Europe and the U.S.

The planet-lab concept was not practical for the Viking Mission, it turned out, but a small mass spectrometer-gas chromatograph will be included in the

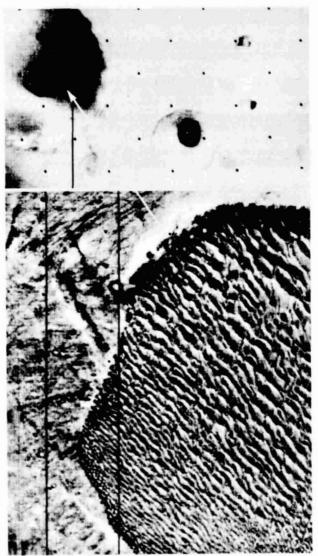
OF POOR QUALITY

This photograph shows evidence of very large-scale depositing by strong winds on Mars. The high-resolution view of the dark region in the center of a large crater shows what very probably are great sand dune fields, similar to those that form in axid areas of Earth. The 30-mile-wide field is composed of numerous long dunes spaced about a mile apart. The straight dark lines are missing lines of picture data.

lander package. The biology package now being developed will include several tests: one for microbe growth in nutrient media; one for decomposition of a radioactive substrate; a test for photosynthesis; a gas metabolism test that will look for changes in the composition of different gases.

HAT IS THE LIKELIHOOD of finding living organisms on Mars with the Viking biology package? "It's feeble. We know so little about the habitat of Mars that Martian bugs would be adapted to. With the limited weight we have for the biology package, and the limited options, it is a big gamble." says Lederberg.

He had noted much earlier that there can never be a simple, unique, definitive detection experiment that can cover all the possible manifestations of life. Indeed, there is no generally accepted definition of life—thus, no single, unambiguous "life detector." But there are instruments that make a few assumptions about the chemical nature of extraterrestrial organisms. Life on



earth is structurally based on carbon and utilizes water as an interaction medium. But must these particular atoms be the atoms of life everywhere, or might there be a wide range of atomic possibilities in organisms on another planet?

"The experiments are not very sensitive," says Lederberg. "They might not work in two weeks. (The biology unit will monitor metabolic activity tests for about two weeks.) It might take three months. Also, the tests will be managed in a strange environment because we have discovered from Mariner 9 that it is more frigid and dry on Mars than we had expected. We found the tests should be carried out at -5 to -10° C, which would be more like the Martian environment. But they have been designed to be carried out at about 10° above zero. So it is really an artificial environment, since we can't devise an experiment now that will withstand freezing temperatures." Engineering constraints prevent such modification at this time.

Lederberg feels the most important experiment may well be the camera system. "The chemical tests may record a negative because of a mismatched environment," he says, "while the camera records shrubbery."

Imagery tests during the past summer established that the camera performance should exceed expectations. Dr. Levinthal, with the other Imagery Team scientists, conducted the first experiment, exercising the stereoscopic mapping capability. The stereoscopic effect is achieved by alternately scanning the same scene from two slightly different angles (two cameras separated by about 0.8 meter). This provides a means of determining the size and distance of objects from the lander. Celor, black and white, and infrared channels were successfully tested on rock powders about the size expected on Mars. Bulk motion and blowing sand could be detected also.

The imaging experiments are expected to contribute to our understanding of the seasonal variations in the surface brightness, and distinctions between bright and dark areas. Cameras will explore the surface for contraction cracks and filling, and will scan the horizon for other planets, satellites, and stars.

There is more concern about another aspect of the Viking Mission. Lederberg recalls that after the great clamor of Sputnik, there was much political interest, but he worried what it meant for scientific progress, and if careful exploration would be overlooked. It was at this stage that he became concerned about contamination—both of Mars and of the earth if a return vehicle should be developed. "I remember talking with officials of the National Academy of Sciences about this, and at first they told me not to worry because planetary investigations would not be looked at in my lifetime. . . ." The Academy has since taken a major role in advising NASA about quarantine precautions.

On the quarantine effort, Lederberg feels that "medi-

cal people are indispensable," since problems involve biological rather than engineering functions.

The Stanford scientists also have been particularly attuned to the problem of spacecraft sterilization and decontamination. A surgeon's scalpel, for example, must be sterile on the outside only. The chance of it disintegrating during a surgical operation are extremely remote. A spacecraft, however, must be completely sterile, at least to the extent of a probability of less than one in a thousand that there will be even a single viable organism anywhere on or within its thousands of component parts. A further matter of concern is the prospect of sacrificing scientific quality because of financial pressure.

As these aspects of the mission grow, Stanford's involvement lessens. Lederberg says that very little, almost none, of the mission-oriented biological work is actually being done at Stanford now. Work here has turned to methodology research in the medical context, applied to patient problems in the mass spectrometergas chromatography labs, to the molecular biology program, and to DNA research. "Exobiology and the Viking Mission to Mars" is also the subject of study for interested students in a freshman seminar which Levinthal is teaching this quarter. The freshmen try their hand at writing the camera program for the lander computer. Levinthal has also taught previous freshman seminars in exobiology.

Finally, after the long research for the mission, do we expect to find Martians? Levinthal likes to talk about this: "We have no reason to think that there are people like us on Mars now . . . the search for intelligent life is another ballgame. We're not looking for civilizations, but for microorganisms."

He explains that most of both the universe we know and life on earth is made of a few particular atoms: carbon, nitrogen, oxygen, hydrogen, helium. Since the atoms most useful for life on earth are also in very high cosmic abundances (those atoms that are needed are just those that happen to be around). Levinthal feels that it is likely there could be enormous numbers of planets in our galaxy with the same behavior in their environments. Others estimate there could be 100,000 to a million civilizations in the Milky Way, an average of a few hundred light-years apart.

Levinthal discusses the idea of life on planets waxing and waning, evolving through millions of years from organic to biological to cognitive activity, and eventually decaying, to form again millions of years hence. Perhaps it is a good time for life now on earth to prosper, but a poor time on Mars . . . perhaps a civilization on Mars decayed some 100,000 years ago . . . "although it is unlikely we will find the signs of a previous civilization, even if it existed," he says, "we will be trying to look for other possible indicators of life. . . . "

—BARBARA BATTINO

Can We Tell Our Doctors What to Be?

The medical profession as a whole must assess the contributions of each specialty and judge where the need is greatest.

by John P. Bunker, M.D.

N A FREE MARKET such as ours in the United States, unplanned and uncontrolled medical manpower results in distribution that does not meet the nation's needs. Up to the very recent past, the medical profession has made no attempt to determine how many physicians are needed in each specialty, nor have our medical schools or teaching hospitals. Clearly, there are acute shortages in some areas, such as general practice and pediatrics; and it is probable that there is an excess of physicians in other specialties. A particularly well known example is that there are more neurosurgeons in Massachusetts for a population of five million than there are in England and Wales for a population of fifty million. But many other specialties also have a relative oversupply of physicians; and indeed, there are overall far too many specialists in comparison to gen-

In the absence of planning, there are identifiable forces which determine the quantity and variety of grad, are specialty (residency) training. The physician, himself, usually has a clear career goal at the time of graduation from medical school or during internship. Having paid for much of his education, he understandably feels free to choose a medical specialty and a place of practice based primarily on his own personal preference.

The young physician's choice is, of course, limited to the spectrum of hospital appointments offered. The appointments offered are determined, in turn, primarily on the basis of the service needs of the hospital. Nowhere does the ultimate national need for trained practitioners enter into the decision.

The service needs of the teaching hospitals are very large. Teaching hospitals provide 25 percent of the hospital care in this country, and they provide a large majority of the highly specialized care, such as kidney dialysis, open heart surgery, and the care of premature infants. It is the young physicians in postgraduate training who provide almost all of the physician manpower and much of the skill necessary to carry out these complicated procedures.

We are training young men and women in a system which is providing highly specialized care. Lots of these, incidentally, are foreign medical graduates. This is hardly the kind of education which prepares one to go out into general practice in this country. This training is even less appropriate to prepare foreign graduates to go back to their own countries. No wonder many of them stay here, and no wonder most of them wind up in specialties.

Undergraduate medical training is not well designed to prepare physicians for primary care. I believe that this is largely the result of the economic structure and financing of our medical schools. Our faculties are made up of highly sophisticated researchers and clinicians whose sources of income are largely research grants and the practice of medicine and, in particular, the practice of the medical specialties.

There is essentially no economic base for the teaching of medical students and specifically for the teaching of primary care. I believe that it is because our medical schools are funded primarily from patient care fees and research grants that our teaching programs do not meet the needs of the country.

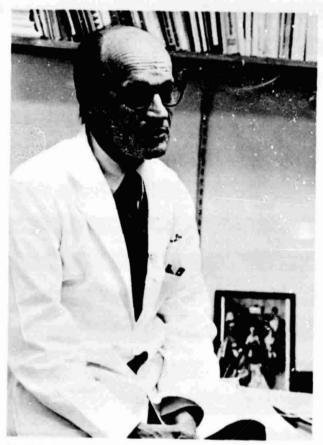
NE MIGHT REASONABLY ANTICIPATE that whatever kinds of doctors are trained, their services will be needed. To a certain extent this is true, for it is now clear that there is an almost unlimited demand for medical services of all varieties. But though demand may be unlimited, our resources, and particularly our physicians, are limited; therefore, it is essential that priorities be established to use these scarce resources and manpower in the most effective and efficient manner possible.

No such comprehensive assessment of medical specialty needs has been made, but one can appreciate the magnitude of the problem by considering the fact that the number of residency positions offered in this country far exceeds the total annual number of medical graduates (of the nearly 17,000 surgical residencies currently offered, only about 12,000 are filled). Many of

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these positions are filled by graduates of foreign medical schools; but it is apparent that the medical specialties could easily absorb all new physicians, leaving none for general practice.

How many specialists should be trained must be determined in light of the needs of a practice of medicine which is in the process of profound reorganization. The education of residents must be geared to the long-range medical needs of the country, and not simply on the basis of the short-term needs of the hospital for an extra pair of hands.



Dr. John P. Bunker, professor of anesthesia, is on sabbatical leave from Stanford and is visiting professor of preventive and social medicine at Harvard Medical School. He served as chairman of the Department of Anesthesia at Stanford for 12 years before stepping down in 1972 to devote his time to research and teaching. In recent years his interests have centered on medical education and health manpower distribution. In 1970 he published a controversial study indicating that the U.S. has too many surgeons and that they do too much unnecessary surgery. Earlier this year, as a Guggenheim Fellow at Harvard, he published a report showing that physicians and their spouses receive as much or more swigery than other professionals and their relatives. Dr. Bunker plans to return to Stanford in July 1975. This article is adapted from testimony on health manpower training before the Senate Health Subcommittee in June, and before the House Committee on Public Health and Environment in May 1974.

The large service element present in medical specialty training is reflected in the fact that interns' and residents' salaries currently are derived primarily from patients' fees. If the primary purpose is to train for the national need, new sources of funds must be found. Dean Robert Ebert of Harvard has suggested that housestaff salaries be paid by the Department of Health, Education, and Welfare, and he further suggests that this "would make it possible to exercise some degree of control over the numbers recruited by various specialties." I believe that *only* when the public pays for medical education can it expect to have a voice in what kinds of physicians are trained and where they practice.

My personal concern with the problems of overspecialization began while I was chairman of the National Halothane Study, a study of anesthetic and surgical deaths sponsored by the National Academy of Sciences ten years ago. One of the most important obcryations of this study was that there were large unexplained differences in postoperative death rates among the 34 participating institutions. This observation suggested that there are large variations in the quality of surgical care; another possible explanation is that there are large differences in the selection of patients—that some hospitals may care primarily for very sick patients with serious surgical illnesses, but that others may specialize in less urgent surgery in good-risk patients.

Data now available strongly suggest that the distribution of surgery may vary widely, and by implication that the indications for which surgery is carried out may also vary. Four years ago I called attention to the fact that there are proportionately twice as many surgeons in the United States as there are in England and Wales, and that they do twice as many operations.1 Within the United States we now know that there are similar large variations in surgical manpowers and in operations. In Kansas² and in Vermont,³ for example, two states for which detailed information is available, there are two, three, and four-fold variations in the rates at which common operations such as tonsillectomy, appendectomy, hysterectomy, and cholecystectomy are performed when one community is compared to another; and it is observed that the highest operation rates occur in communities where there are the most surgeons. (I should point out that operation rates were based on place of residence, thus excluding any effect of travel to medical facilities away from home.)

These data suggest either that some populations receive too much care or that others receive too little, and some observers have drawn the conclusion that there must be a large number of unnecessary operations. But exactly how much surgery is needed for good health is not known, for no one has yet collected the kind of outcome data required for this judgment (data first requested, interestingly, by Florence Nightingale more than 100 years ago).

HAT WE NEED are quantitative regional and national data on the costs, risks, and benefits of surgery. There are already abundant data on the costs of surgery, and a good deal is known concerning the risks, at least in terms of mortality in the immediate postoperative period. But there are relatively few data concerning those who are discharged alive from a hospital. Some die in other hospitals or nursing homes and are lost from the records. (The financial importance of this is suggested by Social Security records which show that as much as 25 percent of Part A hospital Medicare payments are on behalf of patients who are dead a year later.) For those who live, there are virtually no reliable and comprehensive data on how many are relieved of their presenting disability or discomfort, and how many have recovered to their preoperative or preillness level of function; consequently, it is usually not possible to assess the balance of benefits to risks as operation rates are extended to include increasing numbers of marginal procedures.

Such an assessment has been undertaken, however, for one common and important operation, appendectomy. The study was carried out in the Federal Republic of Germany.4 Appendectomy rates were reported to be two to three times higher than those of other countries, and the mortality attributed to appendicitis was also found to be three times as great as in most other countries. Three-quarters of the appendices removed were found to be normal; from this finding, the authors rule out the possibility of an increased prevalence of disease and conclude that the most probable reason for Germany's high mortality rate is that appendectomy is carried out more often than elsewhere. They imply, in short, that when appendectomy is carried out at higher rates for increasingly tenuous indications, the risk of operation eventually exceeds the risk of the disease.

A similar conclusion has recently been drawn by Duncan Neuhauser of the Harvard School of Public Health. On the basis of the mortality of elective herniorrhaphy, the risk of strangulation, and the mortality of emergency operation after strangulation, Neuhauser predicts that in the hands of the average surgeon, the risk of elective herniorrhaphy at the age of 65 or above is four times as great as the risk of not operating. The question that Neuhauser raises is of special interest and importance at present, in view of the fact that the number of herniorrhaphies in the Medicare population has doubled since 1965.

The evidence is strong that some operations are performed with a frequency in excess of documentable cost-benefit usefulness. How general is the phenomenon? Do more total operations actually lead to an increase in overall population mortality? There is some evidence to support this conjecture. In 12.4 Vermont study, Wennberg has observed a positive statistical association between operation rates and overall death

rates, and together with Wennberg, I have recently raised the question as to whether the high operation rates in the United States and Canada may not help to explain the high age-specific mortality rates which are of so much concern on this side of the Atlantic.⁵ Some, but by no means all, of the difference can be attributed to the greater incidence of accidents, homicide, and suicide in this country. Is it possible that some of the remaining difference may be accounted for by the now well-established differences in numbers of discretionary operations?

Discretionary operations all carry a discrete and measurable risk of death. The operative and postoperative mortality for all operations in the United States is approximately 1.4 percent (National Center for Health Statistics, unpublished data). For discretionary operations, one might conservatively assume a mortality of 0.5 percent, which if assigned to operations performed in the United States in excess of those performed in England and Wales, would account for a third to a half of the discrepancy in age-specific death rates.

There are, to date, no national or international published data on postoperative deaths adequate to test this hypothesis. And even if the hypothesis is found to be correct, it does not necessarily mean that fewer operations (than performed in the United States) are better. Death is, after all, only one index of the public health—or lack of it. It is to the improvement in the quality of life—to the relief of disability, discomfort, and disfigurement—that elective surgery is primarily directed. But the costs of surgery, measured in lives as well as dollars, are large, and must be entered into cost-benefit analysis of surgical care.

N ADDRESSING ITSELF to the costs and benefits of surgical care, the medical profession will, first of all, need better data. It will need detailed information on which to estimate and balance the risks of each operation against the risks of nonoperative treatment. For operations such as appendectomy, in which the considerations are largely limited to life and death, the analysis is relatively straightforward. For many discretionary procedures, the question is more difficult to approach; is the likelihood that the operation will lead to a more comfortable, useful, and rewarding life sufficiently great to justify accepting risks of death of one in a thousand, one in a hundred, or greater?

Studies to develop this kind of comprehensive data on cost-effectiveness and risk-benefits are planned, but they will be time-consuming and laborious. Until such data are available, there will continue to be concern that demands for medical and surgical care may exceed need, or, in economic parlance, that the usual checks and balances of the free market do not apply to the medical care "industry." The principal difficulty is, of

course, that the consumer, when he is the average patient, is not an informed consumer. He has no way of telling good medicine from bad, or whether more care is better than less.

In an attempt to circumvent this difficulty, B. W. Brown, professor of biostatistics at Stanford, and I have recently studied physicians as informed consumers of surgical services.⁶ The study was carried out in California and in it we compared physicians and their families with lawyers, ministers, and businessmen. We expected that physicians, cognizant of the risks as well as of the benefits of surgery, would undergo fewer operations. Contrary to our prediction, however, we found that physicians and their families had as many as or more than the other professional groups. Particularly startling was the observation that more than half the wives of male physicians in Santa Clara County will have undergone hysterectomy by the age of 65.

Many of these hysterectomies must have been for convenience, and I suspect that many would be called unnecessary by conventional medical criteria. But to the extent that they were demanded by an informed patient, they must be considered to present a perceived need. I would suggest, however, that some of such surgery is at best a luxury, and, indeed, I suspect that a good deal of surgery is of a luxury variety. With the advent of national health insurance and the removal of financial barriers to medical care, it seems unlikely that we can afford to provide medical and surgical care at this level for all.

In the absence of quantitative data on which to estimate national needs for surgical care, it is difficult to say how many surgeons are needed. We do have some reason, as suggested above, to assume that demands for surgical care by an increasingly informed public will continue to grow.

There are probably already too many surgeons to provide care, even at the current high rates which are obtained in this country, and it is certain that surgeons are being trained at too great a rate. The surgeons themselves now recognize this and have begun to take corrective action. Such self-regulation is a welcome first step, but it is unlikely that this alone will be adequate. I say this simply on the basis that one cannot expect any special interest group voluntarily to make large personal sacrifices.

The medical profession as a whole must assess the relative contribution of each specialty and judge where the need is greatest. To establish administrative mechanisms, and to set priorities, will require a commission broadly representative of the medical profession, the teaching institutions, and the public. The charge to this commission must include the separation of teaching priorities from the needs of hospital service, and therefore must be closely coordinated with total medical manpower planning.

ONCERNING PRIMARY CARE family practice programs, I feel that not only have we not had much support for this in the past, but of course the federal dollar has provided staggeringly large economic incentives in exactly the opposite direction.

The enormous investment in medical research in the 1960's attracted our faculties into tremendously important areas, and we cannot regret the work which wadone. But it was the almost unique emphasis on this aspect which claimed our faculties' attention and, inevitably, the interest of our medical students as well.

What we need to do now is to balance strong basic research support from the government with strong support not just for primary care but for teaching as well. Based on the data provided by the Institute of Medicine and the American Association of Medical Colleges, the cost of teaching a medical student is between \$10,000 and \$26,000 a year, varying with the institution. It also varies with the formula on which the calculation is based. If a student pays for, let us say, \$2,500 or \$3,500 of his tuition, and that is all the school has available to pay for teachers, it is obvious that patient care and research must underwrite much of medical education.

Members of the faculty are only human. They are going to set their own personal priorities on the basis of where their financial support comes from. They are paid primarily to do research and/or care for patients and only secondarily to teach students. As a result, I think the teaching of students is bound to take a back seat.

Many students I have talked with agree with this formulation. Some of my faculty colleagues, on the other hand, seem to think it an unfair assessment, and obviously there are a large number of teachers who love to teach and will do so no matter how much they are paid. But I think we have provided large economic incentives which run counter to the teaching of medical students in some areas such as primary medicine which we now realize are in need.

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The Total Involvement of William Greulich

His work spanned two careers, professor and diplomat, and his search for knowledge ranged around the world.

Is "LABORATORIES" have been U.S. embassies in Britain and Germany; a medical school in Kampala, Uganda; Indian villages; and Hiroshima ruins.

He has served in prestigious foreign capitals as science advisor, directed world-recognized research, and

written an "atlas" that became a handbook in his field.

But Dr. William Walter Greulich says he's had his
most fun and greatest satisfaction teaching medical
students at Stanford.

Attracted by Dr. Charles Haskell Danforth's work in anatomy, Greulich came to Stanford some 43 years ago to begin work for his doctorate and a post as teaching assistant in anatomy. He became professor of anatomy in 1944 and executive head of the department in 1949, then maintained his base at Stanford as his career broadened into foreign and civil service.

The Greulich office today, room 21 of the old adobe and brick Anatomy Building, is the same he occupied in 1931 and returned to through the years. He quietly points out the high ceilings, how cool it is during a hot day, and the former carriage entrance down the hall. If asked, he will describe by name and noteworthy contribution the several framed photographs over his desk—deans, professors, directors of anatomy—recalling them as old friends.

Greulich "retired" from foreign service at the age of 66, and from civil service with the National Institutes of Health at 72. Now 75 years old, emeritus professor of anatomy, he is recognized as an expert on human growth and development. He actively continues his research Monday through Friday at Stanford, and Saturdays in San Francisco at Pacific Medical Center's Institute of Medical Sciences.

After receiving his Ph.D. at Stanford in 1934, Greulich moved to Yale until 1940, first as a Rockefeller Foundation Fellow, and then as assistant and associate professor of anatomy and physical anthropology in the school of medicine.

At Yale he organized and directed the Adolescent Study Unit, initiating a cooperative study by anatomists, physiologists, biochemists, psychologists, and pediatricians. The investigators all studied the same children to focus on the various aspects of human adolescence. During this time Greulich also collaborated with Dr. Herbert Thoms, professor of obstetrics and gynecology at Yale, in various radiographic studies of the skeletal pelvis of both children and adults. Among other things, they found that the shape of the female pelvis has changed markedly since the middle of the 19th century. "The pelvis of the well-circumstanced young woman today is more rounded than that described as the 'normal' female pelvis in the early literature," says Greulich. "The more flattened type was fairly frequent, however, among clinic patients, an observation that suggests that the more flattened pelvis described as 'normal' by early workers was due in part to inadequate nutrition."

Greulich's next step in radiographic studies of skeletal development came with an invitation from Dr. J. Roswell Gallagher to participate in a four-year study of the development of boys at Phillips Academy in Andover, Massachusetts. (Dr. Gallagher, then School Physician, later at Children's Hospital in Boston, established the now widely accepted specialty of adolescent medicine.) Greulich compared the degree of development of the boys' skeletons, determined from repeated radiographs of their hands, wrists, and knees, with their developing sexual characteristics. This work provided the basis for using radiographs of the hand and wrist of children in assessing their general developmental status.

Greulich left New Haven for Cleveland in 1940 to become professor of anatomy and director of the Brush Foundation at Western Reserve University School of Medicine. There he completed the study begun by Professor T. Wingate Todd, examining children from the age of three months, at intervals measuring, weighing, and taking a number of X-rays.

The result of this effort was a major contribution to medical science. Radiographic Atlas of Skeletal Development of the Hand and Wrist. coauthored with Dr. S. Idell Pyle, describes the concept of skeletal age, and with sequences of hand X-rays illustrates how it can be measured. The system came to be known as the Greulich-Pyle Prediction Tables, and is universally used now.

Previously, Greulich notes, terminal stature could

never be known precisely until it had been attained. Inferences from height, weight, and age alone are often not sufficient to judge a child's potential growth. But with X-ray examination of the bones of a child's hand and wrist, a physician can tell how much growth has been completed, since the bones mature in a very specific sequence.

Greulich expanded on this work throughout later years of research. For example, in 1947 while at Stanford he was commissioned to make the first survey of children who survived the atomic bombing of Hiroshima and Nagasaki and to follow their physical growth and development until 1952. Working under the auspices of the National Research Council's Committee on Atomic Casualties, Greulich made a radiographic study of the skeletal development of the hands and wrists of a large number of children in the Hiroshima area, and found that they were retarded in their skeletal development as compared with the children in the U.S. on whom the Greulich-Pyle standards were based.

Greulich discovered scars on the radius of a large percentage of children studied. The scars reflected an interruption of growth believed to have been caused by the irradiation and other injuries which the children incurred at the time of the bombing approximately two years before the films were made.

"We also found that there was a marked sexual difference in the extent to which the growth and development of the Hiroshima and Nagasaki children were adversely affected as a result of the bombing. The boys were more severely retarded than the girls and it took them longer to catch up. This is yet another indication that the female is a much more efficient organism than the male."

Greulich's research on skeletal development was put aside for a time in 1946 when the Carnegie Corporation of New York asked him to visit the medical schools of Australia and New Zealand and to report on ways the corporation might meet some of the research and other postwar needs of those institutions.

post of Science Advisor to the U.S. High Commissioner for Germany. Working with the High Commissioner (and later Ambassador) James B. Conant, former president of Harvard, he surveyed the destruction to schools, libraries, and other institutions and helped implement some of their reconstruction. During his two years there he also served as chairman of the U.S. Educational Commission in the Federal Republic of Germany, usually referred to as the Fulbright Commission. In this capacity, he administered the fellowship program for Americans to study in Germany, and Germans to come to the U.S.

Some years later, in 1961, Greulich left his teaching at Stanford for another foreign service post, this time as Scientific Attaché at the U.S. embassy in London. He describes this as one of the high points in his career. A characteristic *leitmotiv* recurs throughout his work, particularly evident during his London assignment: the importance he places on scientists communicating with one another, whatever their fields of interest or political ties.

"There was one thing we established in those days—and I doubt if it is continued today—which was highly effective. All of the scientific attachés from the various embassies in London would meet together for lunch every month. They included South Africans, Bulgarians, Czechs, Hungarians, Russians, Poles, French, Japanese, and more. We made a pact not to discuss politics. We really got to know one another rather well, and we had very interesting and mutually rewarding sessions together," he says.

The launch of Sputnik spurred the emergence of increased attention on the basic sciences. Greulich recalls a Washington cartoon of the time: "It showed the old scientist being brought up from the cellar into the government office...."

But he also recalls that basic science tended to be forgotten after the furor of Sputnik had passed. "I saw the important benefits of the government agencies making available grants for foreign travel and study to young scientists. Those grants made it possible for young men and women to meet and work with fellow scientists in various parts of the world. The total effort was good for all sides, and had not been possible before the government provided the funds.

"Now, these grants have decreased. Travel fellowships were much more readily available before." He thinks that basic science will continue to be supported from federal funds, but probably at a much reduced rate in the immediate future.

The "brain drain" of British scientists, engineers, physicians, and surgeons into the U.S. was another major topic of diplomatic concern while Greulich was in the London embassy under Ambassador David Bruce. This remains a concern today in many countries, and Greulich foresees it will continue.

In service as senior Fulbright Lecturer at Makerere College Medical School in Kampala, East Africa, Greulich worked at the British Medical Research Council's Laboratory of Infant Malnutrition. He studied, always with the help of his wife, Mildred, children with nutritional diseases, particularly deficiencies created by the lack of protein in their diets.

"In these communities, people regarded a hospital with much fear—as a place where one goes to die. But the mothers began to come with their children, and they would stay with them," he recalls. "This concern is something we might learn from these so-called primitive people... Often the women would be ridiculed by their elders when they returned to their villages,

however, and would discontinue treatments. We learned a lot about the difference between theory and practice there...."

Travels during the Stanford summer breaks also took the Greulichs to Arizona to study the White River Apaches and to New Mexico to study the Pueblo Indians. Greulich notes the "small defect on one finger—kind of an anthropological marker," that indicates the common origin of the Indian and Mongolian populations.

Between assignments in Germany and Britain, Greulich also returned to Stanford classrooms, and to his long-held interest in the Japanese people. He began an extensive study of more than 1,000 children of Japanese ancestry in the Bay Area. He found the children raised here taller, heavier, and more advanced in skeletal development than their counterparts in Japan. This is probably attributable to the more favorable environment in this country, including better hygiene and more varied and adequate diet.

In 1966 another invitation came. This time it was to be assistant to the Director of the Section on Growth and Development at the National Institute of Child Health and Human Development in Bethesda, Maryland. Administration was his chief occupation until he returned to Stanford to complete the NIH study of American-born Japanese he had begun 20 years earlier.

Then, as he puts it, "upon my retirement from NIH, in 1971, I was fortunate enough to be 'reactivated' here at Stanford."

His activity, however, had obviously never slowed. Working with him currently in the Japanese-American so dy is Yoshio Okumoto, Stanford '28, who is marking 50 years with the University. Okumoto worked with Greulich in the early '30s, and assisted in making the photographic copies of the X-ray films for the Atlas. They are now completing a tedious job of locating, interviewing, and measuring the original study subjects, hoping to discover how the early advanced growth affected their development as adults.

Greulich has also long maintained a major interest in twinning, which he attributes to Professor Danforth, who was a recognized authority on that subject.

DESPITE H:S OBSERVATION that "travel is work when you get to be 75," and "spring is not the same in Paris when you're my age," he undertook a vigorous trip (what he called a vacation) to Rome in October to present a paper at the First International Congress of Twin Studies. He then went on to Sofia, Bulgaria, to visit the laboratory of an old friend, then to Belgrade University to see a former colleague, and to London for more visits.

Over the years Greulich has played an active role in several professional societies, serving as first vice-president of the American Association of Anatomists, associ-



ate editor of Anatomical Record, and chairman of the committee on anatomical nomenclature. This group revised the Basle Nomina Anatomica, which since 1895 had been the official list of names and terms used in human anatomy. He also served as president of the American Association of Physical Anthropologists and associate editor of the American Journal of Physical Anthropology. He was president of the Society for Research in Child Development, and is currently completing another term as member of the governing council.

Greulich's work spanned two careers, professor and diplomat, and his travels ranged around the world, yet his desire for new knowledge and his characteristic good humor and diplomacy continue: "Work that one enjoys doing can be a most pleasant and rewarding, though quite incurable, form of addiction. I am grateful to be able to continue to indulge it, especially in the congenial company of so many good friends."

Although the professor no longer teaches young medical students, he grows serious when discussing his former students: "We had some of the best here at Stanford, and it has been very rewarding to see them develop through the years. Many of them have made very substantial contributions, and with very, very few exceptions their professional lives have reflected much credit on Stanford Medical School."

Greulich makes his home at Channing House in Palo Alto, where he likes to keep an eye on the pro football teams as well as Stanford's. Mildred Libby Greulich, his wife of almost 50 years and constant companion and assistant throughout his career, died last year after a long illness. His sons, James, an elementary school printipal in Pasadena, and Richard, research director at the National Institute of Dental Research, are both Stanford '49.

—B.B.

Chief Residents: New Role Models for Women

With more women than ever before in medical schools, women physicians in academic positions offer hope of increased opportunities.

Y RESPONSIBILITIES as chief resident are no different than those of a man in the same position," said Dr. Libby Short, chief resident in medicine at Stanford University Medical Center. "Yet 1 sometimes feel like a closely watched train, carrying the responsibility to do a particularly good job for the sake of the women after me."

With more women than ever before in medical schools throughout the country, female physicians in key academic positions are vital role models in their profession. They provide guidance and encouragement for students, and offer hope of increased opportunities for their sex in the years to come.

Three chief residents for the current year at Stanford University Hospital are women: Dr. Short, who coordinates the activities of some 150 medical housestaff; Dr. Joellen Werne, responsible for about 40 residents in psychiatry, and Dr. Frances Conley, chief of a small core of neurosurgery residents, all men.

They are not the first women to hold such positions at Stanford, and they are not the only ones cutting paths through a field where standards in this country have been largely set by and for men. But each brings an individual perspective to medicine, as a physician and as a woman.

"The female aspect is especially important in psychiatry, where the masculine view dominates and male biases are often reflected." Dr. Werne pointed out. "We need more open and searching commentary by women, and a better understanding of changes in female psychology and women's feelings."

The only female neurosurgeon at Stanford, and one of a handful in the United States, Dr. Conley looks forward to the time when she will no longer be a novelty. "Society thinks of surgeons as men. Yet women are exceptionally versatile, and can make excellent surgeons," she said.

Dr. Short notes that women tend to be more responsive to human issues and to patients' feelings. "Previous social conditioning for 'female' roles works to the advantage of women, particularly as interns and residents," she explains. "Long hours on your feet, taking care of child-like patients in a strange environment, making sure they get tests and medication on time is not unlike organizing a family or getting a five-course meal on the table."

All three women attended medical school in the mid-1960's, when women comprised no more than two percent nationally of all medical students. At times, they admitted, they felt lonely and isolated. But like their classmates of the 1960's, they were bright and confident. None ever experienced overt hostility or encountered any obstacles in the pursuit of medicine.

"It was gratifying, and at the same time frustrating, to be a woman," recalls Dr. Werne, one of six women among 80 graduates of Yale Medical School in 1970. "We had a privileged but inferior status. Everyone knew who we were and paid attention to what we did. On the other hand, we were looked at with more scrutiny and felt more pressure to achieve."

Ever since she can remember, Dr. Short wanted to be a doctor. "I was never discouraged from medicine, but then again I never bothered to ask anyone," she said. At an early age she was sure of herself and able to state her objective. Perhaps it is not surprising she chose internal medicine. "It's a highly verbal field, where one's ability to be rational and present a case clearly is prized."

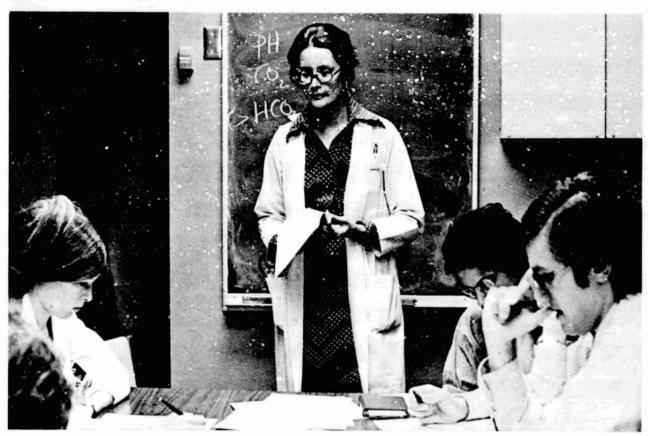
After graduating from Mt. Holyoke College, a wom-

Thirty percent of the new class of Stanford medical students this fall is composed of women, an increase of about four percent from last year.

Fotal enrollment in the Medical School is 389, of which 93 are women.

Women students comprised 12 percent of the firstyear medical students at Stanford in 1966, but that figure fell to three percent in 1969. Since 1970 the number of women students has steadily risen from 10 percent to 26 percent last year.

The national average is 21 percent women first-year medical students. Five years ago the national average was 9.1 percent.



Dr. Libby Short, chief resident in medicine, leads a weekly seminar for medical housestaff and students.

en's school in Massachusetts, Dr. Short married and spent a year in Europe pursuing a favorite interest in weaving. She entered Yale Medical School the following year, and when she received her M.D. in 1968 she became Yale Hospital's first female medical intern in several years. She was a resident and fellow in medicine at Yale and at U.C. San Francisco before coming to Stanford as a senior resident in 1973.

The next goal for Dr. Short to attain is a faculty position. She hopes to do teaching, patient care, and research in hereditary disease and genetics. "Right now it's difficult to be in an academic post without a 'wife' to handle the household and social functions of a busy professional," she explained. "Men in academic medicine run a wild race to reach success by 40, and they are reluctant to acknowledge the possibility of a different timetable. But as more women enter medicine there will be greater role flexibility."

She knows from personal experience that marriage and medicine don't always mix for a woman—after seven years of marriage she is divorced. However, she is thinking of remarrying—this time to a doctor—and does not rule out having a family. "There is nothing wrong with a woman taking a few years from her career to raise children, particularly since women have a longer life expectancy than men anyway," she pointed out. "I am so thoroughly into medicine that i will probably

do it for the rest of my life, but there are five or ten years that I would want to plan very carefully if I had children."

R. Werne's role models for medicine were close to home. Both parents and a brother were in medicine. "With a mother as a practicing pathologist as long as I can remember, I never questioned whether women should be doctors," she said. "It seemed quite natural."

Yet she explored other areas first. At Barnard College in New York she was an English literature major, and began thinking about medicine only toward the end of her undergraduate years. After an additional year of premedical courses and social work in Harlem, she began Yale Medical School. By the time she completed a pediatric internship at Einstein Medical School, Dr. Werne realized she preferred psychiatry, where she could combine her interest in people, sociology, and philosophy. She was a first-year resident in psychiatry at Yale and joined the Stanford psychiatry department in 1973. She, too, leans strongly toward academic medicine. "I love teaching and being in the University environment," she said. "I also enjoy working with patients. There are many women psychiatrists, but few



Dr. Frances Conley, chief resident in neurosurgery, changes the head dressing of one of her patients.

Dr. Joellen Werne, talking with a patient, is responsible for about 40 residents in psychiatry.



are on medical school faculties. We need to relate more closely to other women in the field."

Her career is very important to her, but marriage and family are "viable future possibilities" for Dr. Werne. "I could never stop work, but I could cut down at some period, if I were to have children." Since she would expect to share home responsibilities equally with her husband, it would be practical if he too were in medicine, she added.

Dr. Conley comes from an academic family (her father is a professor at Stanford) which always encouraged the children to be individuals. "As a result, I was never discouraged from becoming a doctor," she said.

She spent two years at Bryn Mawr College, a women's school in Pennsylvania, and then transferred to Stanford. After one year she was admitted to Stanford Medical School. Her graduating class of 1966 included 20 percent women, one of the largest ratios to receive Stanford medical degrees yet. The first female intern in surgery at Stanford Hospital, Dr. Conley had initially considered a career in plastic surgery. However, she was fascinated with her neurosurgery rotation, and so began the grueling but rewarding eight-year residency at Stanford. This is her final year.

Her sex never mattered throughout her training at Stanford, Dr. Conley noted. "Neurosurgery is a small division. Everyone works closely together and there is no room for hard feelings or bias," she explained. "To date, patient acceptance has never been a problem." The real test comes next year, when she may go into private practice. "Then I'll see if physicians are willing to take on a female colleague, and if patients are willing to come to me. Hopefully my skills as a specialist will be appreciated and the role expectation that seems to prevail in general surgery where there are primarily men will not be so great."

Married for 12 years to "a very understanding man," who is not in medicine, Dr. Conley has chosen not to have children. "I feel I can make a more important contribution to society as an M.D. than as a mother. There is no way I could do my work and be a housewife at the same time." Fortunately, she notes, her husband wants a companion, not a "homebody." They share several interests, including long-distance running and flying (both have a pilot's license). "I'm always on call and can't plan ahead for our activities, but I've learned how to organize my days," Dr. Conley said. "When there's something I want to do, I make time to do it." She even has time to serve on the Alumni Association Board of Governors.

The challenge for women in medicine is tremendous, according to Dr. Conley, and she believes that women should meet it in their own style. "Actions speak louder than words," she emphasizes. "Women can pursue a medical career in a feminine and gracious manner that is competent and effective."

—HALI WICKNER

ALUMNI

Hoover Medal Winner

Dr. Russel Van Arsdale Lee, '16, M.D. '20, became the ninth recipient of the Herbert Hoover Medal for Distinguished Service, the highest honor bestowed by the Stanford Alumni Association, at a dinner in his honor August 6.

Founder in 1930 of the Palo Alto Medical Clinic, Dr. Lee was given the medal by John R. Johnson, president of the Stanford Alumni Association, and by University President Richard

W. Lyman.

The Clinic became a model for hundreds of similar medical organizations, and Dr. Lee became a vigorous proponent of group practice. Nationally recognized as an authority on the socioeconomics of medical care, Lee retired in 1960 as clinical professor of medicine at the Medical Center after 42 years as faculty member. He played a key role in the decision to move the School of Medicine from San Francisco

to the campus.

The late President Hoover was first recipient of the medal. Other alumni so honored are J. E. Wallace Sterling, chancellor; John W. Gardner, former Secretary of Health, Education, and Welfare; David Packard, chairman of Hewlett-Packard Co., and former Deputy Secretary of Defense; the late Senator Carl Hayden of Arizona; Frederick Seitz, former president of the National Academy of Sciences and now president of Rockefeller University; Frederick E. Terman, vice-president and provost emeritus of Stanford; and Dorothy Buffum Chandler, founder of the Los Angeles Music Center.

Leland B. Blanchard '37 of San Jose received a commemorative gavel in August as outgoing president of the Society of Teachers of Family Medicine.

George S. Buehler '38 of Whittier is president-elect of the Los Angeles County Medical Association. He will serve in this post until July 1975, when he will be installed as president.

Jesse L. Ray '39 of Portland, Oregon, became president of the United States Section of the International College of Surgeons at their recent seventh Western Hemisphere Congress in Portland. He formerly served as vice-president and president-elect. Edward C. Defoe, Jr. '44B of Shawnee Mission, Kansas, reports he is currently professor and chairman of the Department of Human Ecology at Kansas University Medical School.

Charlotte Umbreit Telesco '44B, who has been living in the Philippines since 1947, was back visiting at the Medical Center during the summer. Having raised five children, she is now considering coming back to the U.S. to practice in the field of geriatrics or with minority groups.

Robert Martin '45 was profiled in an article about Californians in the August issue of PSA magazine. Martin has returned from a three-year stint in Iowa. He is professor and chief of the family practice division at UCLA. He practiced in Concord and Modesto before leaving for Iowa, and had served as president of the California Division of the American Cancer Society and president of the Mount Diablo School Board.

Ralph W. Schaffarzick '46, a San Francisco internist, has been named senior vice-president of Blue Shield of California. He has been associated with Blue Shield since he became medical advisor in 1966, then medical director in 1969.

Augustus A. White III '61, associate professor of orthopedic surgery and

director of the biomechanics research project at Yale University School of Medicine, was presented The Soulville Foundation's youth model award in Los Angeles ceremonies in August. Dr. White has served three years as house physician and youth advisor at a basketball camp for underprivileged boys operated by the foundation at Claremont Men's College,

Harvey Ozer '65 and his wife, Dr. Joy Hochstadt, announce the birth of their first child, a daughter, on August 24 in Worcester, Massachusetts. Both doctors are senior scientists at the Worcester Foundation for Experimental Biology in Shrewsbury, Massachusetts, Ozer is lecturer in genetics at the University of Massachusetts Medical School, and advisory consultant to the National Cancer Institute. His research is in the genetics of somatic ceils and tumor virus-cell interactions. Dr. Hochstadt is adjunct professor of biochemistry at Central New England College. and her research is in the field of cell membrane transport and its relation to cell growth control in cancer.

Gordon W. Keating '67 has opened his office for the practice of adult and child psychiatry in Seattle, Washington. He is a member of the faculty of Transactional Analysis Northwest and the Northwest Family Training Institute.

Medical Center News

Construction Continues

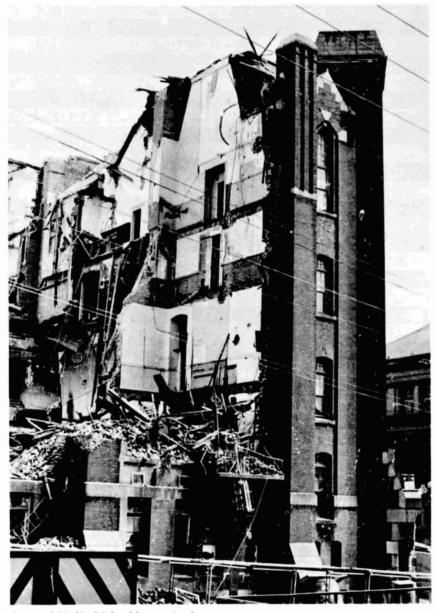
Despite rising costs of materials, the energy crisis, and strikes, construction at the Medical Center is on schedule. Projects include the Hospital core expansion, the Sherman Fairchild Center for the Neurosciences, the Governor's Lane complex, and a renal care unit.

The Hospital expansion will add vitally needed support facilities, including 25 intensive care beds, increased emergency services, radiology, cardiology, surgical pathology, and postanesthesia recovery areas. Initially, University trustees approved a budget of \$9.5 million for this project, but recently revised it to \$12.6 million because of construction and equipment costs. These were primarily caused by

design changes required by state laws for earthquake safety. Even with the increase in budget, the project has been scaled down to only those parts most critical. The facility is tentatively scheduled to be completed and in operation by December 1975.

The Sherman Fairchild Center will include the Neurosciences Building and an adjacent auditorium, located immediately southwest of the Hospital. The Neurosciences Building will be three stories and will house the departments of anatomy, physiology, and neurobiology (a new department). The auditorium will provide seating for 400 persons. The \$9.7 million facility is scheduled to be in operation in January 1976.

Funding is from the Fairchild Foundation, In addition, a \$2 million



Stanford Medical School has received 1,000 of the old bricks from the demolition of the Cooper Medical School Building in San Francisco, A 4,000pound carved granite block, inscribed "Stanford University Medical Department," has also been brought to the campus to be installed as a memorial. Where the artifacts will be housed is still to be determined. The West's first medical college was built in 1882 for \$125,000 by Dr. Levi Cooper Lane. In 1908 Stanford accepted Cooper Medical School as its Department of Medicine, which continued until 1959. The building remained in use by Presbyterian Hospital of Pacific Medical Center for outpatient clinics. administration, and research until June of this year.

gift from Mrs. Frances Nunnally Winzer of La Jolla will permit construction of a third floor in the Center and help complete diagnostic and treatment laboratories in the Hospital addition.

The Governor's Lane complex will be located west of the Medical Center. The facility, supported by the National Cancer Institute with Dr. Henry S. Kaplan as principal investigator, will provide laboratories for the Lymphoma Virology Program of the Department of Radiology. It is scheduled for completion in June 1975, at an expected cost of \$1,155,000.

The :enal care unit, located southwest of the Psychiatry Building, is a new temporary structure, costing approximately \$185,000.

Kretchmer Appointed

Dr. Norman Kretchmer, Harold K. Faber professor of pediatrics at Stanford, has been named director of the National Institute of Child Health and Human Development (NICHD) of the National Institutes of Health.

The pediatrician and biochemist was on sabbatical leave from Stanford

as a Guggenheim Fellow this year, and served as consultant to the office of the NIH director. In his new position he succeeds Dr. Gilbert Woodside, acting director.



As former chairman of the De-

partment of Pediatrics, Kretchmer broadened the scope of clinical and scientific programs, and headed the committee responsible for the University's undergraduate human biology major. Before coming to Stanford in 1959 he was associate professor of pediatrics at Cornell University.

In addition to his new administrative responsibilities, Kretchmer will continue to study the biochemical basis for hereditary and metabolic diseases of infants, and will also be clinical professor of pediatrics at Howard University and consultant to the U.S. Navy at Bethesda National Naval Hospital.

Emergency Paramedics

Twenty-two firemen from Palo Alto and South San Francisco are undergoing 12 weeks of training in the Department of Emergency Services at Stanford to become qualified paramedics, or emergency medical technicians.

The paramedics will treat a patient on the scene while keeping contact by radio with a physician or nurse at the Medical Center until the patient arrives at a hospital.

Switchboard operators at police and fire stations will summon the paramedics when needed. They will not replace ambulance attendants on emergency runs. The paramedics will not transport the patient, although one may accompany the patient on the ride to the hospital. The other member of the two-man team will follow the ambulance in his own emergency van in case the patient should develop complications along the way.

The Palo Alto City Council has set a flat fee of \$40 for each patient, to reduce unnecessary calls. Dr. Michael Eliastam, director of emergency services at Stanford, hopes the program will expand so that the Stanford community will ultimately have its own paramedic unit. Once firemen have completed the training and final testing, including oral examinations, they will be certified by the county public health department.

Rehabilitation Center

The first comprehensive center on the west coast for design and fabrication of devices for physically disabled children has been established at Children's Hospital at Stanford. It is believed to be the first center in the U.S. for research and development of rehabilitation aids for children with neuromuscular impairments.

The center is funded in part by a matching grant of \$800,000 from the Max C. Fleischmann Foundation of Reno, Nevada. It is headed by Maurice LeBlanc, former staff engineer for the committee on prosthetics research and development of the National Academy

of Sciences,

Children referred by physicians will be examined by a team that includes orthopedic surgeons, physical and occupational therapists, and rehabilitation engineers. The device for each child will depend on his stage of development and particular problem. The philosophy today, according to Le-Blanc, is to provide the child with a life-style that includes as normal development as possible. "Instead of giving children a brace and sending them home, we hope to offer them a wardrobe of devices, all tailored to their individual needs and in tune with their growth," he says.

The center is expected to cooperate with the Palo Alto Veterans Administration Hospital, particularly concerning adult patients with spinal cord in-

juries.

Cancer Center Created

The School of Medicine will be part of a major comprehensive cancer center program recently launched in the Bay Area.

The coordinating body of the new Northern California Cancer Program will include the University of California School of Medicine in San Francisco, the American Cancer Society, and Stanford. The center will have no buildings of its own, but will work through existing facilities, including community hospitals. Lay programs of the American Cancer Society also will be coordinated with the plan.



Dr. Eugene Bleck, .enter, chief of orthopedics and rehabilitation at Children's Hospital at Stanford, checks the plastic leg brace of patient Larry Lozares with staff orthotist Wallace Motloch. His braces are similar to the products the new Rehabilitation Engineering Center will fabricate.

The local planning committee is chaired by Dr. Saul A. Rosenberg, professor and head of the division of oncology at Stanford. The committee foresees, in addition to the coordination of basic science and clinical investigation, the expansion of training programs and establishment of new and enlarged cancer detection and resource clinics, consultation services and radiotherapy networks, development of uniform procedures in pathology and epidemiology, and expansion of public education programs.

It is expected that the Northern California Cancer Program will combine all of the present anticancer activities into a coordinated effort involving not only research but also public and professional education and some aspects of service to cancer patients.

The program is expected to be financed in large part by the National Cancer Institute. Sixteen existing centers in operation elsewhere in the nation have budgets ranging from \$700,000 to \$12.6 million, or an average of about \$3 million per year. Participating institutions have provided only seed anoney to get the program going. Once incorporated, the program will apply to NCI for increased working funds.

Brain-scanning Device

The Department of Radiology at Stanford recently placed its new EMI Scanner into operation for examination of patients with suspected brain disorders. It will detect benign and malignant brain tumors, blood clots, brain injuries, brain defects resulting from strokes, and certain birth defects. The scanner may also be used as the takeoff point for research into areas involving actual and potential brain disease.

According to Dr. William Scott, acting assistant professor of radiology, the device will not totally replace tests previously used. It may shorten the hospitalization of some patients, and in others act as a supplement to pneumoencephalograms and angiograms.

The Palo Alto Medical Clinic also operates an EMI Scanner, and, according to Scott, Stanford and the clinic plan to work together on joint clinical research utilizing both scanners. Stanford will also scan patients for smaller hospitals without their own scanners. Scott expects that about a half dozen such devices will be in operation in the near future in the greater Bay Area.

Letters

Medical Missionary

Back in the Stanford M.D., May 1967, you had an article, "Portrait of a Medical Missionary."

Another similar article . . . appeared in the April 1974 number of the Reader's Digest. ["My Most Unforgettable Character," about Dr. Ainslie's long work in Guatemala.]

In both of these articles, my only hope is that they may cause some to pass up the big dollar and concentrate on helping the thousands who have no medical help at present.

Already the Reader's Digest article has caused two ladies to write letters saying, one that she is now a sophomore in medicine and will be on her way the day she is an M.D.; another who is farther away from an M.D. says she has already picked out the Indian town where she will spend her life. . . .

I have always felt honored to be a Stanford Medical School alumnus.

Charles Ainslie, M.D. '16 Guatemala

Tuskegee Study

As Dr. Gibsen ("The Case of Untreated Syphilis," Stanford M.D., Spring 1974) has ably pointed out, there was in 1932 a widespread belief that latent seropositive syphilis should be treated more or less energetically with the then available arsenicals. However, at that time there was no unequivocal proof that the untreated seropositive individual had any shorter

life span than his neighbor. The Macon County population was chosen for the investigation because of the high probability that that population would not receive antisyphilitic therapy without outside intervention. Had not the Public Health Service selected this group for observation their women would also not have received the therapy that they did receive. The Public Health Service was not authorized at that time to select one county for massive treatment of all seropositive persons.

At that time evidence of mesaortitis was found in 40 percent of the black male persons coming to autopsy, as contrasted with 20 percent of white American seamen and 5 or 6 percent of other white males. The seamen, black or white, received vigorous arsenical treatment, or had it available in the early course of their disease. But many of these, of course, had acquired their infection in prearsenical days. Had the Public Health Service not considered the information sought of importance to the public health, no team would have gone into Macon County, and the subjects of the investigation would not have received the benefits that they did receive. I was interested in mesaortitis at the time and wrote a small study of its incidence in American seamen (Venereal Disease Information 15: 39-50, 1934)

I was in NIH in the early thirties and it was then still small enough that we heard most of what was going on, also in field public health. As I remember, one of the inducements was the promise of a free funeral. This was important to these rural blacks, and as I heard it there was actually competition to get included in the study group. I think that the treatment of the women was a later development, and was not actually a part of the study plan.

This I write from an old man's memory. I was not in on the study though I believe I saw some of the early histologic post mortem material. I write as a pathologist, not as a clinician. The study has all the earmarks of a well-planned epidemiologic study designed to elicit the biology of the disease as it occurred in an isolated rural population, without intensive therapy.

In conclusion, I must dissent from Dr. Gibson's condemnation of the Tuskegee study. Would Dr. Gibson have regarded the study any more favorably had it been confined to watching this population age and die, women as well as men, simply providing funerals and having autopsies performed? This was after all a public

health study, not a therapeutic experiment.

R. D. LILLIE, M.D. '20 Medical Director U.S. Public Health Service, Retired

Admissions Policy

I feel profound gratitude to Stanford for my undergraduate, but most especially my medical education. However, I have some personal reservations about current policies and trends at the University and Stanford Medical School. . . .

Medical School Admission Policy:

a) Stanford premeds: More preference should be given to Stanford premeds (or at least some preference) for admission. It's admirable to aspire to a cosmopolitan, heterogeneous student body, but not at the expense of handicapping your own alumni who are exceptionally qualified.

b)Overreliance on GPA and medical aptitude scores: No crush of 5,000-plus applications should justify the current impersonal overutilization of GPA and "Met Cat" numbers to choose a student body. . . .

c) Admission attitude:

I have no formula for choosing the future Osler, but a change in attitude from being in a commanding "seller's position" is needed. A colleague's daughter with a 4.0 GPA and widely accepted to medical schools, was asked for an interview at Stanford; after making a special trip she was told by the interviewer that he didn't know why they bothered to interview her as her aptitude scores were not sufficiently high. . . .

Clearly this authoritarian elitist attitude does Stanford at least as much damage as it does to the individual applicant. No system at all would be better than a solely numerical one for choosing future members of the Stanford family. Whatever system or nonsystem is used, a definite change in attitude is of primary importance.

As a clinician whose heroes were bedside teachers, I believe Stanford Medical School has become too preoccupied with the scientific, technical, and experimental aspect of medicine. By itself such a philosophical change would be justified, but not at the expense of de-emphasizing the individual, clinical, and common sense aspect of medicine. . . .

Let's get back to teaching compassionate commitment, and the only teaching of any generation that students will emulate is teaching by example. . . .

ARTHUR O. WEBER, M.D. '53, B.S. '49 Torrance

[Dr. Clayton Rich responded to Dr. Weber's letter, and his reply follows.—Editor]

Let me speak first about admission procedures. . . . First, we do take more students from Stanford than from any other undergraduate institution. For our class entering this fall, 39 undergraduate colleges were represented. Sixteen students in the class came from Stanford. The school providing the pext largest number of matriculants was Harvard with nine. Yale followed with eight. Then MIT and UC-Berkeley both contributed four. The remainder of the institutions provided either one, two, or three students. Therefore, you can see that we do take almost twice as many students from Stanford as from the schools ranking close to Stanford.

Next, you suspected that there is an overuse of grade point average and Medical College Admission Test scores in decisions concerning the entering class at Stanford. . . . We are concerned about the heavy reliance which is placed on these two indices of potential success in medicine by both those seeking admission and those making the decisions, here and elsewhere. Because of this, our Committee on Admissions spends an extraordinary number of hours interviewing applicants and reviewing folders during which time they look most carefully for personal traits and personal interests that will suggest those with the best potential for the practice of medicine.

Most of the students we accept have grade point averages between 3.5 and 3.8, whereas we turn down hundreds of applicants with grade point averages of 4.0. You would be very impressed, I believe, if you were to sit in on one Committee meeting and witness the deliberations about the highly qualified applicants. Clearly, the thrust of the discussion is in the direction of looking carefully for important personal characteristics.

Of course, these applicants have been previously selected as having a very sound academic background. In this respect, we attemp: to be realistic about GPA and MCAT scores and use them a two elements in the Committee's decision. The test, for example, was designed primarily to identify students who could not intellectually handle the rigors of medical school and it does not distinguish well between a student performing at the 70th percentile and one performing at the 90th percentile.

Clearly, we do admit students who have high GPA and MCAT scores and they come from some of the best undergraduate institutions of this country. We don't believe that this background will exclude students who have a distinctive potential for a balanced practice of medicine. Obviously, because the number of very able applicants exceeds places available here, we will not admit many who will likewise do very well at other medical schools and in the practice of medicine.

John Steward, our associate dean for student affairs and chairman of the Admissions Committee, received his degrees from Stanford (A.B. 1948 and M.D. 1955) as you did. It is his impression that the students being admitted today are more committed to a medical practice which clearly focuses on the patients as individuals than were the students of his day or even ten years ago. It is his strong feeling that current students' interests are broader and that they, in large part, are unmistakably committed to the practice of a compassionate and humanitarian form of medicine. The present students' preferences for internships and residencies in family, pediatric, and obstetric medicine and surgery seem to support Dr. Steward's observations. When all is said and done, about 90 percent of our students enter the practice of medicine.

I also discussed your letter with Dr. William P. Creger who is professor of medicine and associate dean for student affairs. He shares your observation that there was insufficient emphasis in the 1960's on clinical teaching in many of this country's medical schools, including this one. But that condition is being corrected, here and elsewhere. Recent appointments to the faculty and to leadership of the key departments for clinical teaching have given emphasis to this point. The number of clinical clerkships offered by faculty currently exceeds the number of clerkships which the students can take. In Dr. Creger's view, "teaching compassionate commitment to today's students is easy. Our students insist upon it and a rising proportion of faculty enjoy offering such experiences.

CLAYTON RICH, M.D. Vice President for Medical Affairs and Dean of the School of Medicine

Cushing on Full-time

As you may recall, in Fall 1972 Stanford M.D. published a small paper of mine titled "An Alternative to the Full-Time Faculty." This paper was met with a considerable outpouring of letters generally supportive of the position described in the article. Recently at the request of Dr. Richard Warren, editor of the Archives of Surgery, I wrote an editorial along these same lines. The number of requests for reprints would suggest that there is considerable interest in this matter in departments of surgery around the country.

Since these publications, I found an article by Dr. John Green of Phoenix, Arizona, who pointed out several interesting similar attitudes maintained both by Dr. Harvey Cushing, surgeonin-chief of the Peter Bent Brigham, and Dr. Henry Christian, physician-inchief. I should like to quote some of the paragraphs from this article.

"Our self-imposed program, however, was not permitted to stand unchallenged, for in October 1913, after some overtures the General Education Board offered to give the Harvard Medical School \$1,500,000 provided the chiefs of clinic at the Brigham Hospital would agree to serve as full-time officers on the same financial basis as that about to be instituted in Baltimore. The medical school was then in debt and the promised financial relief would have been a veritable windfall, but I could never convince myself that those here who pressed us to accept the restrictions imposed by the gift, apart from several of our preclinical colleagues, were interested as much in the principle involved as securing the gift.

"On my own part, a term of service limited to 20 years, with an academic salary on which a family of children were to be educated and no pension in sight in case of accident or ill health, seemed a dubious proposition. I doubted, moreover, whether such an arrangement would in any way activate me and feared indeed that it might encourage indolence. If the purpose of the plan was to prevent the attendants in university hospitals from exploiting their position for their personal ends, there was just as much reason to fear, human nature being what it is, that hospital superintendents (deans) and trustees might be tempted in a pinch to exploit their salaried professional attendants.

"With no pretense to be anything more than an amateur investigator, I could not see that fixed salary could either improve my status in this respect, or give me any more freedom for such researches as I was capable of directing or undertaking. And coming of a race of general practitioners, the intimate and confidential relation between doctor and patient (one of the most precious things in medicine) was in my blood and I could not look upon the cold institutional program with any great enthusiasm, much less with any expectation that it would serve to make something out of me that I was not already."

Another interesting quote from Dr. Green's paper was ascribed to Dr. Clyde T. Hardy, an obviously enlightened dean from Bowman Grey School of Medicine, who emphasized that "where there is no relationship between income and effort there is a definite danger that practice will be

neglected."

Dr. Hardy quoted John Gardner, a former secretary of HEW, who has pointed out that a four percent increase in physician productivity would in one year add the equivalent of 11,700 physicians to supply America. Hardy comments that Gardner might also have added that a 10 to 15 percent decrease in productivity, which could happen if practicing physicians were forced onto a salary system, would lose us the equivalent of 30,000 to 40,000 physicians.

It is encouraging to many of us who see far more evils in a full-time system for the clinical departments of the medical school than in a geographical system, to find that leading figures in medicine throughout its modern history in the United States have agreed with this point of view, and that the administrative officers who favor a full-time system in its rigid sense do so because it allows them sufficient controls to exploit the working clinical faculty.

Roy Cohn, M.D. '33 Walter Chidester Professor of Surgery

Books

Change, Principles of Problem Formation and Problem Resolution, by Paul Watzlawick, Ph.D., John H. Weakland, Ch.E., and Richard Fisch, M.D., 192 pages. Published by W.W. Norton & Company, N.Y., 1974, 87,95.

This book deals with how problems arise and are perpetuated in some instances, and resolved in others. It examines how common sense and logical approaches often fail or compound a problem, while seemingly illogical and unreasonable actions succeed in producing a desired change.

Authors are concerned with concepts of human communication, interactional therapy, the pathogenic and therapeutic effects of paradoxes, and of action-oriented rather than origin-oriented techniques of problem resolution.

Watzlawick is assistant clinical professor and Fisch clinical instructor, both in the Department of Psychiatry at Stanford. All have private practices in Palo Alto.

Camping Therapy, Its Uses in Psychiatry and Rehabilitation, edited by Thomas Power Lowry, M.D., 138 pages. Published by Charles C. Thomas, Springfield, Illinois, 1974. \$7.75.

This collection of readings on the psychiatric value of camping is intended for therapists, camp directors, and mental health administrators interested in outdoor recreation as a therapeutic tool.

Dr. Lowry and 17 contributors describe a broad range of experiences, including a historical-philosophical overview, discussions on camping therapy applied to psychosis, juvenile delinquency, childhood neuroses, and mental retardation. There is also a perspective essay on man's spiritual relationship with nature. Suggestions are offered for materials and resources for establishing an economical camping therapy program.

Dr. Lowry, class of '57, is in private practice in Corte Madera.

Atlas of Hand Surgery, by Robert A. Chase, M.D., 438 pages. Published by Saunders, Philadelphia, 1973, \$30.

This is an authoritative atlas, illustrated with fine drawings. It includes sections on fingertip injuries, skin and soft tissue losses, amputations, vascular reconstruction, nerve surgery, bone and joint reconstruction, reconstruction of the thumb, surgery of rheumatoid arthritis, and tendons, fascia, and muscles.

DNA Synthesis, by Arthur Kornberg, M.D., 399 pages. Published by W. H. Freeman and Company, San Francisco, 1974. \$18.

Dr. Kornberg, Merner professor of biochemistry at Stanford, presents a comprehensive treatment of DNA synthesis, emphasizing its biochemical rather than physiological aspects. It includes the topics of precursors, repair, recombination, restriction, and transcription.

The book is designed to serve students with a beginning interest in DNA synthesis, as well as those working directly on the subject. It is suitable as a text for graduate courses in molecular biology, biochemistry, cell biology, biophysics, and molecular genetics. Illustrations include colored schemes and electron micrographs.

Principles of Drug Action: The Basis of Pharmacology, 2nd edition, by Avram Goldstein, M.D., Lewis Aronow, Ph.D. and Sumner Kalman, M.D. Published by the Biomedical-Health Division of John Wiley & Sons, 1974. \$21.50.

The second edition of this book is designed as a comprehensive volume for the nonspecialist dealing with general pharmacology.

Authors are all professors of pharmacology at Stanford; Kalman is an alumnus, class of '51.

Discussed are drug receptors and receptor theory, time courses of drug action, onset and disappearance rates of drug effects, environmental poisons and mechanisms of antidotal action against ordinary poisons. The book explains the principles of drug action, absorption, metabolism, distribution, toxicity, and clinical use of drugs and evaluation of drug effects and safety.

The book is organized around systematic principles rather than drug families or types of drug effect. It begins with drugs at the molecular-receptor level, proceeds to clinical trials.

Computer Applications and Techniques in Clinical Medicine, by Herbert R. Ludwig, Ph.D., 317 pages. Published by John Wiley & Sons, Inc., New York, 1974. \$16.95.

Dr. Ludwig introduces fundamental concepts and describes applications he has developed in the use of computer technology in the clinical laboratory.

He describes how to set up generalized medical information systems, files, and records, with applications in blood banking, plastic surgery, and information systems in the medical environment. He also deals with how to retrieve and analyze specific parameters of patient records.

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Dr. Ludwig is assistant professor of industrial engineering at Stanford. Since 1970 he has been associated with the Stanford Computation Center, and has worked on a bone marrow reporting system, a cleft palate retrieval and data analysis system, and an information system for the department of surgery.

The book is a volume in the Stanford series on Methods and Techniques in the Clinical Laboratory, edited by Paul L. Wolf, M.D., formerly of the Stanford faculty.

Primary Care: Where Medicine Fails, edited by Spyros Andreopoulos, 212 pages. Published by John Wiley & Sons, 1974, 89,95.

This new book focuses on the present health care system—defining primary care, analyzing the public's access to it, examining its economics, and determining standards for a better primary care system.

Based on the third symposium of the Sun Valley Forum on National Health, the book includes papers by Dr. Alberta Parker of UC-Berkeley, Dr. Charles Lewis of UCLA, Dr. Stanley S. Bergen, Jr. of the College of Medicine and Dentistry of New Jersey, and Dr. Karen Davis of the Brookings Institution. Discussion by other forum participants is included.

Among the conclusions is that improved primary care should be the federal government's and the medical profession's first priority for the health care delivery system.

Andreopoulos is information officer of Stanford University Medical Center and editor of Stanford M.D.

Deaths

Lloyd Blackwell Dickey, M.D., emeritus professor of pediatries. Died July 18, 1974, in San Rafael at the age of 80. A native of Wisconsin, Dr. Dickey moved to California in the early 1920's to become instructor and later professor at Stanford. He served as acting head of the Department of Pediatrics for three separate terms, and was widely engaged for consultation in pediatrics and tuberculosis in the Bay Area. In a memorial resolution prepared by Drs. Alvin H. Jacobs, Harold K. Faber, Roy B. Cohn, and Robert H. Alway, his associates remember that he was "Pop to his colleagues and friends, and also to student doctors and nurses. . . . His early interest in zoology continued as an avocation with the Audubon Society . . . making him a walking botanical encyclopedia. He is remembered with affection by generations of Stanford medical and nursing students and colleagues."

Hale F. Shirley, M.D., emeritus professor of pediatrics and psychiatry. Died October 7, 1974, at Stanford University Hospital after a long illness. He was 73. A pioneer in developing child psychiatry as a medical specialty. Dr. Shirley was a leader in the field throughout his career. He joined the faculty in 1939 and served as director of the child psychiatry clinic until his

retirement in 1971. He became emeritus professor in 1966. The Hale Shirley Memorial Fund for child psychiatry has been established in the Department of Psychiatry in his honor.

Late notices:

Frank B. Reardan, M.D., Class of 1911, of Sacramento. Died 1974.
Donald G. Davy, M.D., Class of 1920, of Sausalito. Died September 1, 1974.
James H. Hall, M.D., Class of 1922, of Belmont. Died October 6, 1974.
A. Carol McKenney, M.D., Class of 1925, of Pebble Beach. Died September 17, 1974.
Joseph Dwight Wilson, M.D., Class of 1925, of Carmel. Died November 9, 1973.

Cletus S. Sullivan, M.D., Class of

1926, of San Jose. Died May 11, 1974.

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Acrial photograph by Adrian R. Hatheld, Los Altos Hills Stanford Programs Changes